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=> e osteoprotegerin/cn

E1	1	OSTEOPONTIN K (CATTLE CLONE PBK2.1 PRECURSOR REDUCED)/CN
E2	1	OSTEOPONTIN K (OX CLONE PBK2.1 PRECURSOR REDUCED)/CN
E3	1 -->	OSTEOPROTEGERIN/CN
E4	1	OSTEOPROTEGERIN (HUMAN CLONE LAMBDA-OIF10 PRECURSOR)/CN
E5	1	OSTEOPROTEGERIN (HUMAN CLONE PRRCMV-HUOSTEOPROTEGRIN)/CN
E6	1	OSTEOPROTEGERIN (HUMAN GENE OPG PRECURSOR REDUCED)/CN
E7	1	OSTEOPROTEGERIN (HUMAN GENE TR1)/CN
E8	1	OSTEOPROTEGERIN (HUMAN)/CN
E9	1	OSTEOPROTEGERIN (MOUSE CLONE PDSR.ALPHA.-MUOPG)/CN
E10	1	OSTEOPROTEGERIN (MUS MUSCULUS GENE OPG PRECURSOR REDUCED)/CN
E11	1	OSTEOPROTEGERIN (RATTUS NORVEGICUS CLONE PB1.1 GENE OPG PREC URSOR REDUCED)/CN
E12	1	OSTEOPROTEGERIN LIGAND/CN

=> s e3

L1 1 OSTEOPROTEGERIN/CN

=> fil medl,caplus,biosis,embase,wpids;s (l1 or osteoprotegerin or opg) (20w) (bind? protein or receptor)

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FILE 'WPIDS' ENTERED AT 15:22:33 ON 21 MAR 2000

L2 33 FILE MEDLINE
L3 47 FILE CAPLUS
L4 27 FILE BIOSIS
L5 30 FILE EMBASE
L6 2 FILE WPIDS

TOTAL FOR ALL FILES
L7 139 (L1 OR OSTEOPROTEGERIN OR OPG) (20W) (BIND? PROTEIN OR RECEPTOR)

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PROCESSING COMPLETED FOR L7
L8 55 DUP REM L7 (84 DUPLICATES REMOVED)

=> d cbib abs 1-55;s (l1 or psteoptotegerin or opg) and antibod?

L8 ANSWER 1 OF 55 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 1
2000:160180 Osteoprotegerin prevents and reverses hypercalcemia in a murine model of humoral hypercalcemia of malignancy. Capparelli, Casey; Kostenuik, Paul J.; Morony, Sean; Starnes, Charlie; Weimann, Bernadette; Van, Gwyneth; Scully, Sheila; Qi, Meiying; Lacey, David L.; Dunstan, Colin R. (Department of Pathology, Amgen Inc., Thousand Oaks, CA, 91320-1789, USA). Cancer Res., 60(4), 783-787 (English) 2000. CODEN: CNREA8. ISSN: 0008-5472. Publisher: AACR Subscription Office.

AB **Osteoprotegerin** (**OPG**), a novel, secreted tumor necrosis factor **receptor** family member that inhibits osteoclast formation and activity was examd. for its activity in a syngeneic tumor model of humoral hypercalcemia of malignancy. Normal mice bearing Colon-26 tumors develop increases in both parathyroid hormone-related protein (PTHrP) expression and plasma PTHrP, marked hypercalcemia, and increased bone resorption. OPG, given either at the onset of hypercalcemia or after it had occurred, blocked tumor-induced increases in bone resorption and hypercalcemia and rapidly normalized blood ionized calcium. In tumor-bearing mice, OPG treatments reduced osteoclast activity from approx. 2-fold above normal into the subphysiol. range but had no effects on tumor size, tumor-induced cachexia, or PTHrP levels. The potent effects of OPG in this humoral hypercalcemia of malignancy model suggest a potential therapeutic role for OPG in the prevention and treatment of this disorder.

L8 ANSWER 2 OF 55 MEDLINE DUPLICATE 2
2000133237 Document Number: 20133237. Interactions between cancer and bone marrow cells induce osteoclast differentiation factor expression and osteoclast-like cell formation in vitro. Chikatsu N; Takeuchi Y; Tamura Y; Fukumoto S; Yano K; Tsuda E; Ogata E; Fujita T. (Division of Endocrinology, University of Tokyo School of Medicine, Tokyo, 112-8688, Japan.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (2000 Jan 19) 267 (2) 632-7. Journal code: 9Y8. ISSN: 0006-291X. Pub. country:

United States. Language: English.
AB Cancer cells metastasized to bone induce osteoclastogenesis for bone destruction. Coculture of either mouse melanoma B16 or breast cancer Balb/c-MC cells with mouse bone marrow cells (BMCs) induced osteoclast-like cells, which were not observed when cancer cells were segregated from BMCs. Osteoclast differentiation factor (ODF), also known as receptor activator of NF-kappaB ligand (RANKL), is a direct mediator of many osteotropic factors. Neither BMCs, B16 nor Balb/c-MC cells alone expressed ODF mRNA. However, coculture of these cancer cells with BMCs induced ODF expression, which was prevented by indomethacin. Moreover, the coculture with cancer cells inhibited secretion of **osteoprotegerin** /osteoclastogenesis inhibitory factor (**OPG**/OCIF), an inhibitory decoy **receptor** for ODF, from BMCs. Thus, enhanced osteoclastogenesis in the presence of cancer cells might be due to an increase in ODF activity. These results suggest that interactions between cancer cells and BMCs induce ODF expression and suppress OPG/OCIF level in metastatic foci resulting in pathological osteoclastogenesis for bone destruction. Copyright 2000 Academic Press.

L8 ANSWER 3 OF 55 MEDLINE DUPLICATE 3
2000111091 Document Number: 20111091. A tumor necrosis factor decoy receptor homologue is up-regulated in the brook trout (Salvelinus fontinalis) ovary at the completion of ovulation. Bobe J; Goetz F W. (Institut National de la Recherche Agronomique, S.C.R.I.B.E., Campus de Beaulieu, 35042 Rennes Cedex, France.) BIOLOGY OF REPRODUCTION, (2000 Feb) 62 (2) 420-6. Journal code: A3W. ISSN: 0006-3363. Pub. country: United States. Language: English.
AB An up-regulated cDNA fragment was obtained from differential-display polymerase chain reaction of brook trout ovarian tissue stimulated by phorbol-12-myristate-13-acetate (PMA) and calcium ionophore A23187. Using this cDNA as a probe, a full-length cDNA of 2267 base pairs was obtained by screening a library of PMA/A23187-stimulated ovarian cDNA. The mRNA obtained presumably encodes for a 302-amino acid protein showing similarities with several members of the tumor necrosis factor (TNF) receptor superfamily. The protein contains several cysteine-rich domains characteristic of mammalian TNF receptor members and is most similar to human decoy receptor 3 and **osteoprotegerin**, two soluble decoy TNF **receptors**. Consequently, this TNF **receptor** homologue was tentatively named a trout decoy **receptor** (TDcR). On Northern blots of ovarian tissue, TDcR hybridized with a 2.2-kilobase transcript that was strongly up-regulated under phorbol ester stimulation. TDcR mRNA was localized in granulosa cells and was detected in the ovary during and after natural ovulation. Its expression was up-regulated at the end of ovulation and progressively down-regulated after 48 h postovulation. Among other trout tissues tested, the transcript was present only in the testis. To our knowledge this is the first description of a member of the TNF receptor family from a lower vertebrate and the first report of a decoy-like TNF receptor in the vertebrate ovary.

L8 ANSWER 4 OF 55 MEDLINE DUPLICATE 4
2000105352 Document Number: 20105352. Tumor necrosis factor alpha stimulates

osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. Kobayashi K; Takahashi N; Jimi E; Udagawa N; Takami M; Kotake S; Nakagawa N; Kinosaki M; Yamaguchi K; Shima N; Yasuda H; Morinaga T; Higashio K; Martin T J; Suda T. (Department of Biochemistry, School of Dentistry, Showa University, Tokyo 142-8555, Japan.) JOURNAL OF EXPERIMENTAL MEDICINE, (2000 Jan 17) 191 (2) 275-86. Journal code: I2V. ISSN: 0022-1007. Pub. country: United States. Language:
English.

AB Osteoclast differentiation factor (ODF, also called RANKL/TRANCE/OPGL) stimulates the differentiation of osteoclast progenitors of the monocyte/macrophage lineage into osteoclasts in the presence of macrophage colony-stimulating factor (M-CSF, also called CSF-1). When mouse bone marrow cells were cultured with M-CSF, M-CSF-dependent bone marrow macrophages (M-BMM phi) appeared within 3 d. Tartrate-resistant acid phosphatase-positive osteoclasts were also formed when M-BMM phi were further cultured for 3 d with mouse tumor necrosis factor alpha (TNF-alpha) in the presence of M-CSF. Osteoclast formation induced by TNF-alpha was inhibited by the addition of respective antibodies against TNF receptor 1 (TNFR1) or TNFR2, but not by osteoclastogenesis inhibitory factor (OCIF, also called **OPG**, a decoy **receptor** of ODF/RANKL), nor the Fab fragment of anti-RANK (ODF/RANKL **receptor**) antibody. Experiments using M-BMM phi prepared from TNFR1- or TNFR2-deficient mice showed that both TNFR1- and TNFR2-induced signals were important for osteoclast formation induced by TNF-alpha. Osteoclasts induced by TNF-alpha formed resorption pits on dentine slices only in the presence of IL-1alpha. These results demonstrate that TNF-alpha stimulates osteoclast differentiation in the presence of M-CSF through a mechanism independent of the ODF/RANKL-RANK system. TNF-alpha together with IL-1alpha may play an important role in bone resorption of inflammatory bone diseases.

L8 ANSWER 5 OF 55 MEDLINE

2000155530 Document Number: 20155530. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Gravallese E M; Manning C; Tsay A; Naito A; Pan C; Amento E; Goldring S R. (Beth Israel Deaconess Medical Center, New England Baptist Bone and Joint Institute, and Harvard Medical School, Boston, Massachusetts, USA.) ARTHRITIS AND RHEUMATISM, (2000 Feb) 43 (2) 250-8. Journal code: 90M. ISSN: 0004-3591. Pub. country: United States. Language: English.

AB OBJECTIVE: Osteoclast differentiation factor (ODF; also known as **osteoprotegerin** ligand, **receptor** activator of nuclear factor kappaB ligand, and tumor necrosis factor-related activation-induced cytokine) is a recently described cytokine known to be critical in inducing the differentiation of cells of the monocyte/macrophage lineage into osteoclasts. The role of osteoclasts in bone erosion in rheumatoid arthritis (RA) has been demonstrated, but the exact mechanisms involved in the formation and activation of osteoclasts in RA are not known. These studies address the potential role of ODF and the bone and marrow microenvironment in the pathogenesis of osteoclast-mediated bone erosion in RA. METHODS: Tissue sections from the bone-pannus interface at sites of bone erosion were examined for the presence of osteoclast precursors by the colocalization of messenger RNA (mRNA) for tartrate-resistant acid phosphatase (TRAP) and cathepsin K in mononuclear cells. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to identify mRNA

for ODF in synovial tissues, adherent synovial fibroblasts, and activated T lymphocytes derived from patients with RA. RESULTS: Multinucleated cells expressing both TRAP and cathepsin K mRNA were identified in bone resorption lacunae in areas of pannus invasion into bone in RA patients. In addition, mononuclear cells expressing both TRAP and cathepsin K mRNA (preosteoclasts) were identified in bone marrow in and adjacent to areas of pannus invasion in RA erosions. ODF mRNA was detected by RT-PCR in whole synovial tissues from patients with RA but not in normal synovial tissues. In addition, ODF mRNA was detected in cultured adherent synovial fibroblasts and in activated T lymphocytes derived from RA synovial tissue, which were expanded by exposure to anti-CD3. CONCLUSION: TRAP-positive, cathepsin K-positive osteoclast precursor cells are identified in areas of pannus invasion into bone in RA. ODF is expressed by both synovial fibroblasts and by activated T lymphocytes derived from synovial tissues from patients with RA. These synovial cells may contribute directly to the expansion of osteoclast precursors and to the formation and activation of osteoclasts at sites of bone erosion in RA.

L8 ANSWER 6 OF 55 CAPLUS COPYRIGHT 2000 ACS

2000:120320 Development of Disulfide Peptide Mapping and Determination of Disulfide Structure of Recombinant Human Osteoprotegerin Chimera Produced in Escherichia coli. Merewether, Lee Anne; Le, John; Jones, Michael D.; Lee, Richard; Shimamoto, Grant; Lu, Hsieng S. (Department of Protein Structure, Amgen Inc., Thousand Oaks, CA, 91320, USA). Arch. Biochem. Biophys., 375(1), 101-110 (English) 2000. CODEN: ABBIA4. ISSN: 0003-9861. Publisher: Academic Press.

AB Recombinant human osteoprotegerin chimera is a 90-kDa protein contg. a human IgG Fc domain fused to human osteoprotegerin. The mol. is a dimer linked by two intermol. disulfide bonds and contains eleven intramol. disulfide bonds per monomer. A cysteine-rich region in **osteoprotegerin** contains nine disulfide bridges homologous to the cysteine-rich signature structure of the tumor necrosis factor **receptor**/nerve growth factor **receptor** superfamily. In this report, we have developed peptide mapping procedures suitable to generate disulfide-contg. peptides for disulfide structure assignment of the fusion mol. The methods employed included proteolytic digestion using endoproteinases Glu-C and Lys-C in combination followed by LC-MS analyses. Disulfide linkages of peptide fragments contg. a single disulfide bond were assigned by sequence anal. via detection of (phenylthiohydantoinyl) cystine and/or by MS anal. Disulfide bonds of a large, core fragment contg. three peptide sequences linked by four disulfides were assigned after generation of smaller disulfide-linked peptides by a secondary thermolysin digestion. Disulfide structures of peptide fragments contg. two disulfide bonds were assigned using matrix-assisted laser desorption ionization mass spectrometry with postsource decay. Both the inter- and intramol. disulfide linkages of the chimeric dimer were confirmed. (c) 2000 Academic Press.

L8 ANSWER 7 OF 55 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

2000055312 EMBASE Osteoprotegerin and osteoprotegerin ligand mediate the local regulation of bone resorption. Dunstan C.R.. Dr. C.R. Dunstan, M/S 27-5-A, Amgen Inc., Thousand Oaks, CA 91320, United States. cdunstan@amgen.com. Endocrinologist 10/1 (18-26) 2000. Refs: 55.
ISSN: 1051-2144. CODEN: EDOCEB. Pub. Country: United States. Language: English. Summary Language: English.

AB Osteoblasts and marrow stromal cells are known to mediate the bone resorptive effects of osteotropic hormones and cytokines. The mechanism by

which osteoblasts and stromal cells regulate osteoclastogenesis and osteoclast activation has been elusive. Recent discoveries have elucidated a signaling pathway that satisfies the requirements for this mediation. **Osteoprotegerin** ligand (OPGL), RANK, and **osteoprotegerin** (**OPG**) form a signal (agonist), **receptor**, and decoy **receptor** (antagonist) triad. OPG was discovered first as an inhibitor of osteoclastogenesis in vivo and in vitro. OPGL was identified by its binding affinity to OPG and is a potent stimulator of osteoclast differentiation, activation, and survival, in the absence of stromal cells. OPGL was found to be identical to TRANCE, which has been identified as a ligand for a tumor necrosis factor (TNF) receptor family member named RANK. RANK has been confirmed as the appropriate receptor for the effects of OPGL on bone resorption. Gene deletion studies in mice have shown that OPG is essential to maintain normal bone mass in mice and OPGL is essential for osteoclast formation. Osteotropic hormones and cytokines regulate OPG and OPGL expression in osteoblasts and marrow stromal cells to increase the OPGL signal for bone resorption.

L8 ANSWER 8 OF 55 MEDLINE DUPLICATE 5

2000111577 Document Number: 20111577. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. Hofbauer L C; Khosla S; Dunstan C R; Lacey D L; Boyle W J; Riggs B L. (Endocrine Research Unit, Mayo Clinic, Rochester, Minnesota, USA.) JOURNAL OF BONE AND MINERAL RESEARCH, (2000 Jan) 15 (1) 2-12. Ref: 96. Journal code: 130. ISSN: 0884-0431. Pub. country: United States. Language: English.

AB Although multiple hormones and cytokines regulate various aspects of osteoclast formation, the final two effectors are osteoprotegerin ligand (OPG-L)/osteoclast differentiation factor (ODF), a recently cloned member of the tumor necrosis factor superfamily, and macrophage colony-stimulating factor. **OPG**-L/ODF is produced by osteoblast lineage cells and exerts its biological effects through binding to its **receptor**, osteoclast differentiation and activation receptor (ODAR)/receptor activator of NF-kappa B (RANK), on osteoclast lineage cells, in either a soluble or a membrane-bound form, the latter of which requires cell-to-cell contact. Binding results in rapid differentiation of osteoclast precursors in bone marrow to mature osteoclasts and, at higher concentrations, in increased functional activity and reduced apoptosis of mature osteoclasts. The biological activity of **OPG**-L/ODF is neutralized by binding to **osteoprotegerin** (**OPG**)/osteoclastogenesis inhibitory factor (OCIF), a member of the TNF-**receptor** superfamily that also is secreted by osteoblast lineage cells. The biological importance of this system is underscored by the induction in mice of severe osteoporosis by targeted ablation of OPG/OCIF and by the induction of osteopetrosis by targeted ablation of OPG-L/ODF or overexpression of OPG/OCIF. Thus, osteoclast formation may be determined principally by the relative ratio of OPG-L/ODF to OPG/OCIF in the bone marrow microenvironment, and alterations in this ratio may be a major cause of bone loss in many metabolic disorders, including estrogen deficiency and glucocorticoid excess. That changes in but two downstream cytokines mediate the effects of large numbers of upstream hormones and cytokines suggests a regulatory mechanism for osteoclastogenesis of great efficiency and elegance.

L8 ANSWER 9 OF 55 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 6

1999:464074 Document No. 131:83999 Mammalian receptor protein NTR-5 and cDNA
and methods of diagnosis and therapy. Valenzuela, David M. (Regeneron Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 9933967 A2 19990708, 27 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US27688 19981228. PRIORITY: US 1997-68925 19971229.

AB Mouse and human cDNAs encoding protein NTR-5, a putative receptor related to **osteoprotegerin** and tumor necrosis factor **receptor**, are disclosed. The invention also provides assay systems that may be used to detect and/or measure ligands that bind the mammalian NTR-5 gene product. The present invention also provides for diagnostic and therapeutic methods based on the interaction between mammalian NTR-5 and agents that initiate signal transduction through binding to mammalian NTR-5.

L8 ANSWER 10 OF 55 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 7

1999:355802 Document No. 131:15462 Receptor **OPG**-2 member of the tumor necrosis factor **receptor** family and its diagnostic and therapeutic applications. Tschopp, Jurg (Biogen, Inc., USA). PCT Int. Appl. WO 9926977 A1 19990603, 22 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US25065 19981124. PRIORITY: US 1997-66446 19971124; US 1998-74896 19980217.

AB The cDNA and deduced amino acid sequence of a novel human receptor in the tumor necrosis factor receptor family, designated OPG-2, is provided. OPG-2 is identified based on its sequence similarity to the published sequence of OPG-1. OPG-2 is useful (no data) in the treatment of osteopenic disorders such as those characterized by excessive osteoclast activity such as primary osteoporosis, Paget-s disease of the bone, hypercalcemia of malignancy, and osteolytic metastases.

L8 ANSWER 11 OF 55 MEDLINE DUPLICATE 8

1999427835 Document Number: 99427835. Stimulation of osteoprotegerin ligand and inhibition of osteoprotegerin production by glucocorticoids in human osteoblastic lineage cells: potential paracrine mechanisms of glucocorticoid-induced osteoporosis [see comments]. Hofbauer L C; Gori F; Riggs B L; Lacey D L; Dunstan C R; Spelsberg T C; Khosla S. (Endocrine Research Unit, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905, USA.) ENDOCRINOLOGY, (1999 Oct) 140 (10) 4382-9. Journal code: EGZ. ISSN: 0013-7227. Pub. country: United States. Language: English.

AB Osteoporosis is a serious complication of systemic glucocorticoid use. However, while glucocorticoids increase bone resorption in vitro and in vivo, the mechanism(s) of this effect are at present unclear. Recent studies have identified the **osteoprotegerin** (**OPG**) ligand (**OPG**-L) as the final effector of osteoclastogenesis, an action that is opposed by the soluble neutralizing **receptor**, OPG. Thus, we assessed glucocorticoid regulation of OPG and OPG-L in various human osteoblastic lineage cells using Northern analysis, RT-PCR, and ELISA. Dexamethasone inhibited constitutive OPG messenger RNA (mRNA)

steady-state levels by 70-90% in primary (MS) and immortalized stromal cells (hMS), primary trabecular osteoblasts (hOB), immortalized fetal osteoblasts (hFOB), and osteosarcoma cells (MG-63). In hFOB cells, dexamethasone inhibited constitutive OPG mRNA steady-state levels in a dose- and time-dependent fashion by 90%, and also suppressed cytokine-stimulated OPG mRNA steady-state levels. Dexamethasone-induced inhibition of OPG mRNA levels was not affected by the protein synthesis inhibitor, cycloheximide, and was shown to be due to inhibition of OPG gene transcription using a nuclear run-on assay. Moreover, dexamethasone also dose dependently (10(-10) M-10(-7) M) inhibited constitutive OPG protein concentrations in the conditioned medium of hFOB cells from 2.59 +/- 0.02 ng/ml (control) to 0.30 +/- 0.01 ng/ml (88% inhibition; P < 0.001 by ANOVA). Concurrently, dexamethasone stimulated OPG-L mRNA steady-state levels in MS and hFOB cells by 2- and 4-fold, respectively. Treatment of murine marrow cultures with conditioned medium harvested from dexamethasone-treated MG-63 cells increased tartrate-resistant acid phosphatase (TRAP) activity by 54% (P < 0.005) compared with medium harvested from control-treated cells (in the presence of OPG-L and macrophage colony-stimulating factor). Moreover, dexamethasone (10(-8) M) promoted osteoclast formation in vitro, as assessed by a 2.5-fold increase of TRAP activity in cell lysates (P < 0.001) and the appearance of TRAP-positive multinucleated cells. Our data are thus consistent with the hypothesis that glucocorticoids promote osteoclastogenesis by inhibiting OPG and concurrently stimulating OPG-L production by osteoblastic lineage cells, thereby enhancing bone resorption.

L8 ANSWER 12 OF 55 MEDLINE DUPLICATE 9
1999392980 Document Number: 99392980. Estrogen stimulates gene expression and protein production of osteoprotegerin in human osteoblastic cells. Hofbauer L C; Khosla S; Dunstan C R; Lacey D L; Spelsberg T C; Riggs B L. (Mayo Clinic, Rochester, Minnesota 55905, USA.) ENDOCRINOLOGY, (1999 Sep) 140 (9) 4367-70. Journal code: EGZ. ISSN: 0013-7227. Pub. country: United States. Language: English.

AB The identity of the paracrine mediator(s) of the antiresorptive action of estrogen on bone cells is controversial. **Osteoprotegerin** (**OPG**) was recently identified as a soluble member of the tumor necrosis factor (TNF) **receptor** (TNF-R) superfamily that is secreted by osteoblast lineage cells and acts by binding to and neutralizing its cognate ligand, OPG-L, a required factor for osteoclastogenesis. OPG prevents bone loss when administered to ovariectomized rats, induces osteoporosis when ablated in knock-out mice, and induces osteopetrosis when overexpressed in transgenic mice. In conditionally immortalized, human osteoblastic hFOB/ER-3 and hFOB/ER-9 cell lines containing physiological concentrations of approximately 800 and approximately 8,000 functional estrogen receptors (ER)/nucleus, respectively, we found that 17beta-estradiol dose- and time-dependently increased OPG mRNA and protein levels to maximal levels of 370% and 320%, respectively (P < 0.001); co-treatment with the "pure" antiestrogen ICI 182,780 abrogated these effects completely. 17beta-Estradiol also dose-dependently increased OPG mRNA and protein levels in normal human osteoblasts with approximately 400 ER/nucleus by 60% and 73%, respectively. Thus, estrogen enhancement of OPG secretion by osteoblastic cells may play a major role in the antiresorptive action of estrogen on bone.

L8 ANSWER 13 OF 55 MEDLINE DUPLICATE 10
1999360593 Document Number: 99360593. Parathyroid hormone stimulates TRANCE

and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: correlation with osteoclast-like cell formation. Lee S K; Lorenzo J A. (V.A. Connecticut Healthcare System, Newington 06111, USA.) ENDOCRINOLOGY, (1999 Aug) 140 (8) 3552-61. Journal code: EGZ. ISSN: 0013-7227. Pub. country: United States. Language:
English.

AB We studied the effects of PTH on the expression of tumor necrosis factor-related activation-induced cytokine (TRANCE), **osteoprotegerin** (**OPG**), and **receptor** activator of NF kappaB (RANK) messenger RNA (mRNA) in cultured murine bone marrow, calvaria, and osteoblasts. TRANCE, OPG, and RANK are recently identified regulators of osteoclast formation. Bone marrow cells were cultured with or without PTH(1-34) for 6 days. TRANCE, OPG, and RANK mRNA were measured by RT-PCR. In 6-day cultures, PTH stimulated the number of OCL/well in a dose-dependent manner. A time course showed significant ($P < 0.01$) increases in OCL/well after 24 h of PTH (100 ng/ml). TRANCE mRNA expression, like OCL formation, increased dose dependently and was maximal, with 10-100 ng/ml PTH. In contrast, OPG mRNA expression was decreased by 0.1 ng/ml PTH (40%) and completely abolished by 1 ng/ml. TRANCE mRNA expression was rapidly stimulated by PTH (maximal response at 1 h, 8.1-fold over control). Expression declined by 40% at 24 h but was still much greater than control at 6 days (4.6-fold) in a time-course study. PTH caused a transient stimulation of OPG mRNA at 1 h (2-fold), which returned to basal levels by 2 h. After 6 h, PTH completely inhibited OPG mRNA. There were only minor effects of PTH on RANK mRNA expression. PTH had less potent effects on TRANCE and OPG mRNA expression in calvaria organ cultures and osteoblasts. In mouse calvaria cultures, TRANCE expression was detectable in controls and was increased 2.9-fold by PTH at 24 h. PTH treatment of calvaria decreased OPG expression by 30% at 6 h. MC3T3 E-1 osteoblastic cells expressed minimal levels of TRANCE mRNA either before or after PTH treatment. OPG mRNA was present in MC3T3 E-1 cells, but levels were not modulated by PTH. In primary osteoblastic cells, PTH stimulated TRANCE mRNA expression 4-fold at 2 h and inhibited OPG mRNA expression by 46%. These results demonstrate a tight correlation between the ability of PTH to stimulate OCL formation in marrow culture and expression of TRANCE ($r = 0.87$, $P < \text{or} = 0.05$) and OPG mRNA ($r = -0.88$, $P < \text{or} = 0.05$). Reciprocal regulation of TRANCE and OPG mRNA by PTH preceded its effects on OCL formation by 18-23 h. Hence, it is likely that PTH regulates bone resorption, at least in part, via its effects on TRANCE and OPG expression.

L8 ANSWER 14 OF 55 CAPLUS COPYRIGHT 2000 ACS

1999:374161 Document No. 131:143364 Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Hsu, Hailing; Lacey, David L.; Dunstan, Colin R.; Solovyev, Irina; Colombero, Anne; Timms, Emma; Tan, Hong-Lin; Elliott, Gary; Kelley, Michael J.; Sarosi, Ildiko; Wang, Ling; Xia, Xing-Zhong; Elliott, Robin; Chiu, Laura; Black, Tabitha; Scully, Sheila; Capparelli, Casey; Morony, Sean; Shimamoto, Grant; Bass, Michael B.; Boyle, William J.
(Department of Cell Biology, Amgen, Inc., Thousand Oaks, CA, 91320-1799, USA). Proc. Natl. Acad. Sci. U. S. A., 96(7), 3540-3545 (English) 1999. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.

AB A receptor that mediates osteoprotegerin ligand (OPGL)-induced osteoclast differentiation and activation has been identified via genomic anal. of a primary osteoclast precursor cell cDNA library and is identical to the tumor necrosis factor receptor (TNFR) family member RANK. The RANK mRNA was highly expressed by isolated bone marrow-derived osteoclast progenitors and by mature osteoclasts in vivo. Recombinant OPGL binds specifically to RANK expressed by transfected cell lines and purified osteoclast progenitors. Transgenic mice expressing a sol. RANK-Fc fusion protein have severe osteopetrosis because of a redn. in osteoclasts, similar to OPG transgenic mice. Recombinant RANK-Fc binds with high affinity to OPGL in vitro and blocks osteoclast differentiation and activation in vitro and in vivo. Furthermore, polyclonal Ab against the RANK extracellular domain promotes osteoclastogenesis in bone marrow cultures suggesting that RANK activation mediates the effects of OPGL on the osteoclast pathway. Thus, OPGL-induced osteoclastogenesis is directly mediated via RANK on osteoclast precursor cells.

L8 ANSWER 15 OF 55 MEDLINE DUPLICATE 11

1999234089 Document Number: 99234089. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Lomaga M A; Yeh W C; Sarosi I; Duncan G S; Furlonger C; Ho A; Morony S; Capparelli C; Van G; Kaufman S; van der Heiden A; Itie A; Wakeham A; Khoo W; Sasaki T; Cao Z; Penninger J M; Paige C J; Lacey D L; Dunstan C R; Boyle W J; Goeddel D V; Mak T W. (Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 2S2.) GENES AND DEVELOPMENT, (1999 Apr 15) 13 (8) 1015-24. Journal code: FN3. ISSN: 0890-9369. Pub. country: United States. Language: English.

AB Bone resorption and remodeling is an intricately controlled, physiological process that requires the function of osteoclasts. The processes governing both the differentiation and activation of osteoclasts involve signals induced by **osteoprotegerin** ligand (OPGL), a member of tumor necrosis factor (TNF) superfamily, and its cognate **receptor** RANK. The molecular mechanisms of the intracellular signal transduction remain to be elucidated. Here we report that mice deficient in TNF receptor-associated factor 6 (TRAF6) are osteopetrotic with defects in bone remodeling and tooth eruption due to impaired osteoclast function. Using in vitro assays, we demonstrate that TRAF6 is crucial not only in IL-1 and CD40 signaling but also, surprisingly, in LPS signaling. Furthermore, like TRAF2 and TRAF3, TRAF6 is essential for perinatal and postnatal survival. These findings establish unexpectedly diverse and critical roles for TRAF6 in perinatal and postnatal survival, bone metabolism, LPS, and cytokine signaling.

L8 ANSWER 16 OF 55 MEDLINE DUPLICATE 12

1999242608 Document Number: 99242608. The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts. Burgess T L; Qian Y; Kaufman S; Ring B D; Van G; Capparelli C; Kelley M; Hsu H; Boyle W J; Dunstan C R; Hu S; Lacey D L. (Department of Mammalian Cell Molecular Biology, Amgen Inc., Thousand Oaks, California 91320-1789, USA.. tburgess@amgen.com) . JOURNAL OF CELL BIOLOGY, (1999 May 3) 145 (3) 527-38. Journal code: HMV. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB Osteoprotegerin (OPG) and OPG-ligand (OPGL) potently inhibit and stimulate, respectively, osteoclast differentiation (Simonet, W.S., D.L. Lacey, C.R. Dunstan, M. Kelley, M.-S. Chang, R. Luethy, H.Q. Nguyen, S.

Wooden, L. Bennett, T. Boone, et al. 1997. Cell. 89:309-319; Lacey, D.L., E. Timms, H.-L. Tan, M.J. Kelley, C.R. Dunstan, T. Burgess, R. Elliott, A. Colombero, G. Elliott, S. Scully, et al. 1998. Cell. 93: 165-176), but their effects on mature osteoclasts are not well understood. Using primary cultures of rat osteoclasts on bone slices, we find that OPGL causes approximately sevenfold increase in total bone surface erosion. By scanning electron microscopy, OPGL-treated osteoclasts generate more clusters of lacunae on bone suggesting that multiple, spatially associated cycles of resorption have occurred. However, the size of individual resorption events are unchanged by OPGL treatment. Mechanistically, OPGL binds specifically to mature OCs and rapidly (within 30 min) induces actin ring formation; a marked cytoskeletal rearrangement that necessarily precedes bone resorption. Furthermore, we show that antibodies raised against the OPGL receptor, RANK, also induce actin ring formation. OPGL-treated mice exhibit increases in blood ionized Ca++ within 1 h after injections, consistent with immediate OC activation in vivo. Finally, we find that OPG blocks OPGL's effects on both actin ring formation and bone resorption. Together, these findings indicate that, in addition to their effects on OC precursors, OPGL and **OPG** have profound and direct effects on mature OCs and indicate that the OC **receptor**, RANK, mediates OPGL's effects.

L8 ANSWER 17 OF 55 MEDLINE DUPLICATE 13

1999250831 Document Number: 99250831. Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. Yano K; Tsuda E; Washida N; Kobayashi F; Goto M; Harada A; Ikeda K; Higashio K; Yamada Y. (Research Institute of Life Science, Snow Brand Milk Products Co., Ltd, Tochigi, Japan.) JOURNAL OF BONE AND MINERAL RESEARCH, (1999 Apr) 14 (4) 518-27. Journal code: 130. ISSN: 0884-0431. Pub. country: United States. Language: English.

AB **Osteoprotegerin** (**OPG**)/osteoclastogenesis inhibitory factor (OCIF) is a soluble member of the tumor necrosis factor **receptor** family of proteins and plays an important role in the negative regulation of osteoclastic bone resorption. Whether OPG/OCIF circulates in human blood and how its level changes under pathological conditions is not known. To address these issues, a panel of monoclonal antibodies was generated against recombinant OPG/OCIF and screened for reactivity with solid-phase monomeric and homodimeric forms of the recombinant protein. Antibodies that showed high affinity for both forms of OPG/OCIF and those that selectively recognized the homodimer were identified, enabling development of two types of sensitive enzyme-linked immunosorbent assay (ELISA): one that detects both forms of OPG/OCIF equally and one specific for the homodimer. Characterization of circulating OPG/OCIF with these ELISAs revealed that the protein exists in human serum mainly in the monomeric form. The serum concentration of OPG/OCIF increased with age in both healthy Japanese men and women, and was significantly higher in postmenopausal women with osteoporosis than in age-matched controls. Within the osteoporotic group, serum OPG/OCIF concentrations were higher in patients with low bone mass. Serum OPG/OCIF concentrations were also significantly increased in those postmenopausal women with a high rate of bone turnover, as determined by increased serum

bone-specific alkaline phosphatase and urinary excretion of pyridinoline and deoxypyridinoline. The results suggested that circulating OPG/OCIF levels are regulated by an age-related factor(s) and that the increased serum concentration may reflect a compensative response to enhanced osteoclastic bone resorption and the resultant bone loss rather than a cause of osteoporosis.

L8 ANSWER 18 OF 55 MEDLINE DUPLICATE 14

2000039728 Document Number: 20039728. Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. Udagawa N; Takahashi N; Jimi E; Matsuzaki K; Tsurukai T; Itoh K; Nakagawa N; Yasuda H; Goto M; Tsuda E; Higashio K; Gillespie M T; Martin T J; Suda T. (Department of Biochemistry, School of Dentistry, Showa University, Tokyo, Japan.) BONE, (1999 Nov) 25 (5) 517-23. Journal code: ASR. ISSN: 8756-3282. Pub. country: United States. Language: English.

AB We previously reported that osteoblasts/stromal cells are essentially involved in the activation as well as differentiation of osteoclasts through a mechanism involving cell-to-cell contact between osteoblasts/stromal cells and osteoclast precursors/osteoclasts. Osteoclast differentiation factor (ODF, also called RANKL/OPGL/TRANCE) and macrophage colony-stimulating factor (M-CSF, also called CSF-1) are two essential factors produced by osteoblasts/stromal cells for osteoclastogenesis. In other words, osteoblasts/stromal cells were not necessary to generate osteoclasts from spleen cells in the presence of both ODF/RANKL and M-CSF. In the present study, we examined the precise roles of ODF/RANKL and M-CSF in the activation of osteoclasts induced by calvarial osteoblasts. Osteoclasts were formed in mouse bone marrow cultures on collagen gel-coated dishes in response to a soluble form of ODF/RANKL (sODF/sRANKL) and M-CSF, and recovered by collagenase digestion.

When recovered osteoclasts were further cultured on plastic dishes, most of the osteoclasts spontaneously died within 24 h. Osteoclasts cultured for 24 h on dentine slices could not form resorption pits. Addition of sODF/sRANKL to the recovered osteoclasts markedly enhanced their survival and pit-forming activity. M-CSF similarly stimulated the survival of osteoclasts, but did not induce their pit-forming activity. When primary mouse osteoblasts were added to the recovered osteoclasts, resorption pits were formed on dentine slices. Bone-resorbing factors such as 1alpha,25-dihydroxyvitamin D3, parathyroid hormone, or prostaglandin E2 enhanced pit-forming activity of osteoclasts only in the presence of osteoblasts. M-CSF-deficient osteoblasts prepared from op/op mice similarly enhanced pit-forming activity of osteoclasts. The pit-forming activity of osteoclasts induced by sODF/sRANKL or osteoblasts was completely inhibited by simultaneous addition of **osteoprotegerin** /osteoclastogenesis inhibitory factor, a decoy **receptor** of ODF/RANKL. Primary osteoblasts constitutively expressed ODF/RANKL mRNA, and its level was upregulated by treatment with 1alpha,25-dihydroxyvitamin D3, parathyroid hormone, and prostaglandin E2. These results, obtained by using an assay system that unequivocally assesses osteoclast activation, suggest that ODF/RANKL but not M-CSF mediates osteoblast-induced pit-forming activity of osteoclasts, and that bone-resorbing factors stimulate osteoclast activation through upregulation of ODF/RANKL by osteoblasts/stromal cells.

L8 ANSWER 19 OF 55 MEDLINE DUPLICATE 15

1999182310 Document Number: 99182310. A new member of tumor necrosis factor

ligand family, ODF/OPGL/TRANCE/RANKL, regulates osteoclast differentiation
and function. Takahashi N; Udagawa N; Suda T. (Department of Biochemistry,
School of Dentistry, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo, 142-8555, Japan.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1999 Mar 24) 256 (3) 449-55. Ref: 55. Journal code: 9Y8.
ISSN: 0006-291X. Pub. country: United States. Language: English.

AB Osteoclasts, the multinucleated giant cells that resorb bone, develop from monocyte-macrophage lineage cells. Osteoblasts or bone marrow stromal cells have been suggested to be involved in osteoclastic bone resorption. The recent discovery of new members of the tumor necrosis factor (TNF) receptor-ligand family has elucidated the precise mechanism by which osteoblasts/stromal cells regulate osteoclast differentiation and function. Osteoblasts/stromal cells express a new member of the TNF-ligand family "osteoclast differentiation factor(ODF)/**osteoprotegerin** ligand (OPGL)/TNF-related activation-induced cytokine (TRANCE)/ **receptor** activator of NF-kB ligand (RANKL)" as a membrane associated factor. Osteoclast precursors which possess RANK, a TNF receptor family member, recognize ODF/OPGL/TRANCE/RANKL through cell-to-cell interaction with osteoblasts/stromal cells, and differentiate into osteoclasts in the presence of macrophage colony-stimulating factor. Mature osteoclasts also express RANK, and their bone-resorbingactivity is also induced by ODF/OPGL/TRANCE/RANKL which osteoblasts/stromal cells possess. **Osteoprotegerin** (**OPG**)/osteoclastogenesis inhibitory factor (OCIF)/TNF **receptor**-like molecule 1 (TR1) is a soluble decoy **receptor** for ODF/OPGL/TRANCE/RANKL. Activation of NF-kB and c-Jun N-terminal kinase through the RANK-mediated signaling system appears to be involved in differentiation and activation of osteoclasts. Copyright 1999 Academic Press.

L8 ANSWER 20 OF 55 CAPLUS COPYRIGHT 2000 ACS

1999:341715 Document No. 131:153772 Osteoclastogenesis inhibitory factor: role of a novel TNF-like factor (osteoclast differentiation factor). Takahashi, Naoyuki (Department of Dentistry, Showa University, Japan). Clin. Calcium, 9(4), 445-451 (Japanese) 1999. CODEN: CLCCEJ. ISSN: 0917-5857. Publisher: Iyaku Janarusha.

AB A review with 30 refs., on osteoblast/stroma cells in regulation of osteoclast differentiation; discovery and function of ospeoprotegerin (**OPG**) and oteoclastogenesis inhibitory factor (OCIF); discovery and function of osteoclast differentiation factor; and **receptor** activator of NF-.kappa.B-mediated signals in regulation of osteoclast differentiation and function.

L8 ANSWER 21 OF 55 MEDLINE DUPLICATE 16

1999316334 Document Number: 99316334. Osteoclast differentiation factor acts as a multifunctional regulator in murine osteoclast differentiation and function. Jimi E; Akiyama S; Tsurukai T; Okahashi N; Kobayashi K; Udagawa N; Nishihara T; Takahashi N; Suda T. (Department of Biochemistry, School of Dentistry, Showa University, Tokyo, Japan.) JOURNAL OF IMMUNOLOGY, (1999 Jul 1) 163 (1) 434-42. Journal code: IFB. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Osteoclast differentiation factor (ODF), a novel member of the TNF ligand family, is expressed as a membrane-associated protein by osteoblasts/stromal cells. The soluble form of ODF (sODF) induces the differentiation of osteoclast precursors into osteoclasts in the presence

of M-CSF. Here, the effects of sODF on the survival, multinucleation, and pit-forming activity of murine osteoclasts were examined in comparison with those of M-CSF and IL-1. Osteoclast-like cells (OCLs) formed in cocultures of murine osteoblasts and bone marrow cells expressed mRNA of RANK (receptor activator of NF-kappaB), a receptor of ODF. The survival of OCLs was enhanced by the addition of each of sODF, M-CSF, and IL-1. sODF, as well as IL-1, activated NF-kappaB and c-Jun N-terminal protein kinase (JNK) in OCLs. Like M-CSF and IL-1, sODF stimulated the survival and multinucleation of prefusion osteoclasts (pOCs) isolated from the coculture. When pOCs were cultured on dentine slices, resorption pits were formed on the slices in the presence of either sODF or IL-1 but not in that of M-CSF. A soluble form of RANK as well as **osteoprotegerin** /osteoclastogenesis inhibitory factor, a decoy **receptor** of ODF, blocked OCL formation and prevented the survival, multinucleation, and pit-forming activity of pOCs induced by sODF. These results suggest that ODF regulates not only osteoclast differentiation but also osteoclast function in mice through the receptor RANK.

L8 ANSWER 22 OF 55 CAPLUS COPYRIGHT 2000 ACS
1999:372115 Document No. 131:28062 Mechanism for control of differentiation and maturation of osteoclast. Yasuda, Hisataka; Shima, Nobuyuki; Nakagawa, Nobuyuki; Tsuda, Eisuke (Inst. Life Sci., Snow Brand Milk Products Co., Ltd., Japan). Kagaku to Seibutsu, 37(6), 359-361 (Japanese) 1999. CODEN: KASEAA. ISSN: 0453-073X. Publisher: Gakkai Shuppan Senta.
AB A review with 10 refs., on (1) discovery and cloning of osteoclastogenesis inhibitory factor (OCIF, also called **osteoprotegerin**) and osteoclast differentiation factor (ODF), and (2) functions of M-CSF, ODF, ODF **receptor** (RANK), and OCIF in the regulation of differentiation and maturation of osteoclast.

L8 ANSWER 23 OF 55 MEDLINE DUPLICATE 17
1999297164 Document Number: 99297164. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Suda T; Takahashi N; Udagawa N; Jimi E; Gillespie M T; Martin T J. (Department of Biochemistry, School of Dentistry, Showa University, Tokyo, Japan.. suda@dent.showa-u.ac.jp) . ENDOCRINE REVIEWS, (1999 Jun) 20 (3) 345-57. Ref: 83. Journal code: EIK. ISSN: 0163-769X. Pub. country: United States. Language: English.
AB Osteoblasts/stromal cells are essentially involved in osteoclast differentiation and function through cell-to-cell contact (Fig. 8). Although many attempts have been made to elucidate the mechanism of the so-called "microenvironment provided by osteoblasts/stromal cells," (5-8) it has remained an open question until **OPG** and its binding molecule were cloned. The serial discovery of the new members of the TNF **receptor**-ligand family members has confirmed the idea that osteoclast differentiation and function are regulated by osteoblasts/stromal cells. RANKL, which has also been called ODF, TRANCE, or OPGL, is a member of the TNF ligand family. Expression of RANKL mRNA in osteoblasts/stromal cells is up-regulated by osteotropic factors such as 1 alpha, 25(OH)2D3, PTH, and IL-11. Osteoclast precursors express RANK, a TNF receptor family member, recognize RANKL through cell-to-cell interaction with osteoblasts/stromal cells, and differentiate into pOCs in the presence of M-CSF. RANKL is also involved in the survival and fusion of pOCs and activation of mature osteoclasts. **OPG**, which has

also been called OCIF or TR1, is a soluble **receptor** for RANKL and acts as a decoy **receptor** in the RANK-RANKL signaling system (Fig. 8). In conclusion, osteoblasts/stromal cells are involved in all of the processes of osteoclast development, such as differentiation, survival, fusion, and activation of osteoclasts (Fig. 8). Osteoblasts/stromal cells can now be replaced with RANKL and M-CSF in dealing with the whole life of osteoclasts. RANKL, RANK, and OPG are three key molecules that regulate osteoclast recruitment and function. Further studies on these key molecules will elucidate the molecular mechanism of the regulation of osteoclastic bone resorption. This line of studies will establish new ways to treat several metabolic bone diseases caused by abnormal osteoclast recruitment and functions such as osteopetrosis, osteoporosis, metastatic bone disease, Paget's disease, rheumatoid arthritis, and periodontal bone disease.

L8 ANSWER 24 OF 55 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

1999403187 EMBASE The effect of vitamin D on osteoblasts and osteoclasts.

Ikeda K.; Ogata E.. Dr. K. Ikeda, Department of Geriatric Research, Natl. Inst. for Longevity Sciences, 36-3 Gengo, Morioka, Obu, Aichi 474-8522, Japan. kikeda@nils.go.jp. Current Opinion in Orthopaedics 10/5 (339-343) 1999.

Refs: 35.

ISSN: 1041-9918. CODEN: COORE. Pub. Country: United States. Language: English. Summary Language: English.

AB Vitamin D, when administered for severe vitamin D deficiency or in large doses, stimulates osteoclastic bone resorption, presumably through a molecular interaction between recently identified receptor activator of NF- .kappa.B ligand/**osteoprotegerin** ligand on marrow stromal cells and **receptor** activator of NF-.kappa.B on osteoclast precursors. In contrast, small pharmacologic doses of active vitamin D that do not cause hypercalcemia, when administered in vitamin D-sufficient animals with high bone turnover, can suppress bone resorption while maintaining or even stimulating bone formation. In patients with severe vitamin D deficiency, the anabolic effect of vitamin D is mediated primarily through increased serum calcium, whereas active vitamin D can stimulate bone formation in vitamin D-replete animals, independently of serum calcium or parathyroid hormone level. Therefore, the effects of vitamin D on bone depend on its dosage and on the conditions under which it is administered.

L8 ANSWER 25 OF 55 MEDLINE DUPLICATE 18

2000046448 Document Number: 20046448. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Kong Y Y; Feige U; Sarosi I; Bolon B; Tafuri A; Morony S; Capparelli C; Li J; Elliott R; McCabe S; Wong T; Campagnuolo G; Moran E; Bogoch E R; Van G; Nguyen L T; Ohashi P S; Lacey D L; Fish E; Boyle W J; Penninger J M. (Amgen Institute, Toronto, Ontario, Canada.) NATURE, (1999 Nov 18) 402 (6759) 304-9. Journal code: NSC. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Bone remodelling and bone loss are controlled by a balance between the tumour necrosis factor family molecule **osteoprotegerin** ligand (OPGL) and its decoy **receptor** osteoprotegerin (OPG). In addition, OPGL regulates lymph node organogenesis, lymphocyte development and interactions between T cells and dendritic cells in the immune system. The OPGL receptor, RANK, is expressed on chondrocytes, osteoclast precursors and mature osteoclasts. OPGL expression in T cells is induced by antigen receptor engagement, which suggests that activated T cells may

influence bone metabolism through OPGL and RANK. Here we report that activated T cells can directly trigger osteoclastogenesis through OPGL. Systemic activation of T cells in vivo leads to an OPGL-mediated increase in osteoclastogenesis and bone loss. In a T-cell-dependent model of rat adjuvant arthritis characterized by severe joint inflammation, bone and cartilage destruction and crippling, blocking of OPGL through osteoprotegerin treatment at the onset of disease prevents bone and cartilage destruction but not inflammation. These results show that both systemic and local T-cell activation can lead to OPGL production and subsequent bone loss, and they provide a novel paradigm for T cells as regulators of bone physiology.

L8 ANSWER 26 OF 55 MEDLINE DUPLICATE 19

1999423319 Document Number: 99423319. Interleukin-1beta and tumor necrosis factor-alpha, but not interleukin-6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. Hofbauer L C; Lacey D L; Dunstan C R; Spelsberg T C; Riggs B L; Khosla S. (Endocrine Research Unit, Mayo Clinic and Mayo Foundation, Rochester, MN 55905, USA.) BONE, (1999 Sep) 25 (3) 255-9. Journal code: ASR. ISSN: 8756-3282. Pub. country: United States. Language: English.

AB Recent studies have identified **osteoprotegerin** ligand (**OPG**-L) as the essential factor required for osteoclastogenesis, and that the effects are prevented by its soluble **receptor**, osteoprotegerin (OPG). However, there are limited data at present on the regulation of OPG-L expression in human osteoblastic cells by other cytokines. Because interleukin (IL)-1beta, tumor necrosis factor (TNF)-alpha, and IL-6 all increase osteoclastogenesis, we assessed whether OPG-L mRNA steady-state levels were regulated by these cytokines in human osteoblastic cells. By northern analysis, IL-1beta (5 nmol/L) and TNF-alpha (9 nmol/L) increased OPG-L mRNA steady-state levels by up to two- to three-fold in normal marrow stromal cells (MS), an immortalized marrow stromal cell line (hMS), and the osteosarcoma cell line, MG-63, whereas IL-6 (2 nmol/L, with or without its soluble receptor) had no effect on OPG-L mRNA levels in any of these cells. IL-1beta and TNF-alpha increased OPG-L mRNA steady-state levels in the normal MS cells and the hMS cell line in a time- and dose-dependent fashion by up to 4.1-fold and up to 2.6-fold, respectively. Our data are thus consistent with the hypothesis that the proinflammatory and bone-resorbing cytokines, IL-1beta and TNF-alpha, but not IL-6, may stimulate osteoclastogenesis by inducing the expression of OPG-L.

L8 ANSWER 27 OF 55 CAPLUS COPYRIGHT 2000 ACS

1999:307595 Document No. 130:332975 Role of osteoclast differentiation factor, the new member of the TNF ligand family, in osteoclast differentiation and function. Takahashi, Naoyuki; Udagawa, Nobuyuki; Suda, Tatsuo (Sch. Dent., Showa Univ., Tokyo, 142-8555, Japan). Seikagaku, 71(4), 241-253 (Japanese) 1999. CODEN: SEIKAQ. ISSN: 0037-1017. Publisher: Nippon Seikagakkai.

AB A review with 66 refs., on mol. basis of role of osteoblast/stroma cells regulating osteoclast differentiation and function, focusing on osteoclast differentiation factor (ODF), a member of TNF ligand family, expressed by osteoblast/stroma cells, **osteoprotegerin** (**OPG**) /osteoclastogenesis inhibitory factor (OCIF) as a sol. decoy **receptor** that interferes with action of the ODF, and RANK (**receptor** activator of NF-.kappa.B), an essential signaling receptor for ODF.

L8 ANSWER 28 OF 55 BIOSIS COPYRIGHT 2000 BIOSIS
1999:509087 Document No.: PREV199900509087. Osteoprotegerin ligand and osteoprotegerin: Novel implications for osteoclast biology and bone metabolism. Hofbauer, Lorenz C. (1). (1) Endocrine Research Unit, Division of Gastroenterology and Endocrinology, Zentrum fuer Innere Medizin, Philips University, Baldingerstrasse, D-35033, Marburg Germany. European Journal of Endocrinology, (Sept., 1999) Vol. 141, No. 3, pp. 195-210. ISSN: 0804-4643. Language: English.

L8 ANSWER 29 OF 55 CAPLUS COPYRIGHT 2000 ACS
1999:92645 New insight into molecular targets for the regulation of bone mass.
Boyle, William J. (Department of Cell Biology, Amgen, Inc., Thousand Oaks, CA, 91320, USA). Book of Abstracts, 217th ACS National Meeting, Anaheim, Calif., March 21-25, MEDI-132. American Chemical Society: Washington, D. C. (English) 1999. CODEN: 67GHA6.
AB Mol. genetic, cell biol., and biochem. evidence all indicate that osteoprotegerin (OPG), OPGL (TRANCE/RANKL) and ODAR (RANK) interact during key regulatory events that control bone metab. in vivo. OPGL binds is a potent osteoclast differentiation factor that stimulates osteoclastogenesis from bone marrow precursor cells, and subsequently the resoprtion of bone by mature, activated osteoclasts. OPGL interacts with tow proteins; ODAR/RANK, an osteoclast surface receptor that mediates osteoclastogenic signal transduction, and **OPG**, a sol. decoy **receptor** that neutralizes OPGL, and blocks osteoclast development and activation. Together these three protein constitute the key elements required for the pos. and neg. regulation of bone d.

L8 ANSWER 30 OF 55 MEDLINE DUPLICATE 20
1999349896 Document Number: 99349896. A novel molecular mechanism modulating osteoclast differentiation and function. Yasuda H; Shima N; Nakagawa N; Yamaguchi K; Kinosaki M; Goto M; Mochizuki S I; Tsuda E; Morinaga T; Udagawa N; Takahashi N; Suda T; Higashio K. (Research Institute of Life Science, Snow Brand Milk Products Co. Ltd., Tochigi, Japan.. fvbd7042@mb.infoweb.ne.jp) . BONE, (1999 Jul) 25 (1) 109-13. Journal code: ASR. ISSN: 8756-3282. Pub. country: United States. Language: English.
AB Osteoclasts, the multinucleated giant cells that resorb bone, develop from hematopoietic cells of the monocyte/ macrophage lineage. Osteoblasts, as well as bone marrow stromal cells, support osteoclast development through a mechanism of cell-to-cell interaction with osteoclast progenitors. We recently purified and molecularly cloned osteoclastogenesis inhibitory factor (OCIF), which was identical to **osteoprotegerin** (**OPG**). **OPG**/OCIF, a secreted member of the tumor necrosis factor (TNF) **receptor** family, inhibited differentiation and activation of osteoclasts. A single class of high-affinity binding sites for OPG/OCIF appeared on a mouse bone marrow stromal cell line, ST2, in response to 1alpha,25-dihydroxyvitamin D3 [1,25(OH)2D3] and dexamethasone (Dex). When the binding sites were occupied by OPG/OCIF, ST2 cells failed to support the osteoclast formation from spleen cells. To identify an OPG/OCIF ligand, we screened a cDNA expression library of ST2 cells treated with 1,25(OH)2D3 and Dex using OPG/OCIF as a probe. The cloned molecule was found to be a member of the membrane-associated TNF ligand family, and it induced osteoclast formation from mouse and human osteoclast progenitors in the presence of macrophage colony-stimulating

factor (M-CSF) in vitro. Expression of its gene in osteoblasts/stromal cells was up-regulated by osteotropic factors, such as 1,25(OH)2D3, prostaglandin E2 (P(GE2), parathyroid hormone (PTH), and interleukin (IL)-11. A polyclonal antibody against this protein, as well as OPG/OCIF, negated not only the osteoclastogenesis induced by the protein, but also bone resorption elicited by various osteotropic factors in a fetal mouse long bone culture system. These findings led us to conclude that the protein is osteoclast differentiation factor (ODF), a long sought-after ligand that mediates an essential signal to osteoclast progenitors for their differentiation into active osteoclasts. Recent analyses of ODF receptor demonstrated that RANK, a member of the TNF receptor family, is the signaling receptor for ODF in osteoclastogenesis, and that **OPG** /OCIF acts as a decoy **receptor** for ODF to compete against RANK. The discovery of ODF, OPG/OCIF, and RANK opens a new era in the investigation of the regulation of osteoclast differentiation and function.

L8 ANSWER 31 OF 55 MEDLINE DUPLICATE 21

1999165566 Document Number: 99165566. Recent advances in bone biology provide insight into the pathogenesis of bone diseases. Boyce B F; Hughes D E; Wright K R; Xing L; Dai A. (Department of Pathology, University of Texas Health Science Center, San Antonio 78284-7750, USA.) LABORATORY INVESTIGATION, (1999 Feb) 79 (2) 83-94. Ref: 131. Journal code: KZ4. ISSN: 0023-6837. Pub. country: United States. Language: English.

AB Bone is modeled during embryonic development by endochondral and membranous ossification and is continuously remodeled thereafter under the influence of local and systemic factors to provide structural support and assist in calcium homeostasis. Recent studies of knockout and transgenic mice have increased understanding of the regulation of bone modeling during development and of remodeling of mature bone and have shed new light on the pathogenesis of a number of bone disorders. For example, fibroblast growth factor receptor-3, parathyroid hormone-related protein, and tartrate-resistant acid phosphatase affect the function of chondrocytes during endochondral ossification (the latter two by regulating their life spans and thus growth plate thickness and bone length). Some ubiquitously expressed genes seem unexpectedly to have unique functions that are largely confined to bone cells: M-CSF, C-Fos, PU.1, and NF-kappaB are required for osteoclast formation, whereas c-Src and Mitf (microphthalmia transcription factor) are required for osteoclast activity after the cells have formed. Knockout of these genes results in osteopetrosis, a disorder characterized by persistence in marrow cavities of unresorbed osteocartilaginous matrix and, as in some affected humans, by increased mortality. Some proteins seem to act as negative regulators of bone cell function, for example **osteoprotegerin** (a soluble TNF **receptor**) in osteoclasts; osteocalcin, bone sialoprotein, and 5-lipoxygenase in osteoblasts. Regulation of osteoclast life span may be an important mechanism by which estrogen and bisphosphonates prevent bone loss in conditions characterized by increased bone resorption, such as postmenopausal osteoporosis. The unique requirement of bone cells for certain gene products raises the possibility that these cells may have specific responses to inhibitory or stimulatory agents, and that signaling molecules in these response pathways could be specific targets for novel therapies to treat or prevent common bone diseases.

L8 ANSWER 32 OF 55 CAPLUS COPYRIGHT 2000 ACS

1998:728561 Document No. 130:506 Fusion proteins of **osteoprotegerin** dimerization domains and members of the tumor necrosis factor **receptor** family. Boyle, William J.; Wooden, Scott (Amgen Inc.,

USA). PCT Int. Appl. WO 9849305 A1 19981105, 92 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US8631 19980429. PRIORITY: US 1997-850188 19970501.

AB Fusion proteins of an **osteoprotegerin** dimerization domain and a second protein, preferably another member of the tumor necrosis factor **receptor** (TNFR) family are described. These proteins have uses in drug screening and in the treatment of disorders assocd. with members of the TNFR family or their ligands. These fusion proteins may lack cytoplasmic or transmembrane domains allowing them to remain sol. Also provided are nucleic acids encoding the polypeptides, expression vectors and host cells for their prodn. and pharmaceutical compns. comprising the polypeptides. A gene for a fusion protein of tumor necrosis factor-binding protein (TNF-bp) and the dimerization domain was constructed by std. methods and expressed in CHO cells. The fusion protein was more effective than a fusion protein of TNF-bp and Fc domains at protecting L929 cells from killing by tumor necrosis factor .alpha..

L8 ANSWER 33 OF 55 CAPLUS COPYRIGHT 2000 ACS

1998:712352 Document No. 129:328897 A protein binding osteoprotegerin playing a role in osteoclast maturation for use in the treatment of bone loss. Boyle, William J. (Amgen Inc., USA). PCT Int. Appl. WO 9846751 A1 19981022, 108 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US7584 19980415. PRIORITY: US 1997-842842 19970416; US 1997-880855 19970623.

AB An **osteoprotegerin-binding protein** (OPG) involved in osteoclast maturation has been identified based upon its affinity for osteoprotegerin. The protein is a member of the tumor necrosis factor family. A cDNA encoding the protein has been cloned and characterized for use in manuf. of the protein. The protein may be of use in the treatment of bone diseases such as osteoporosis, bone loss due to arthritis or metastasis, hypercalcemia, and Paget's disease (no data). Receptors for **osteoprotegerin-binding proteins** are also described. The receptors, and agonists and antagonists thereof, may be used to treat bone diseases. Amouse OPG cDNA was cloned by screening an expression bank from 32D cells in COS-7 cells with an osteoprotegerin fusion protein with Fc. The mouse cDNA was used to probe a multiple human tissue Northern blot and the mRNA was detected in lymph nodes and fetal liver but in no other tissues. The mouse cDNA was used as a probe to clone a human cDNA. Manuf. of the C-terminal tumor necrosis factor-like .alpha. domain of mouse OPG in Escherichia coli is described. This domain strongly stimulated osteoclast differentiation from stromal cells in culture in a dose-dependent manner. This domain also induced bone resorption in mice in a dose-dependent manner using blood ionized calcium as an indicator. The hematopoietic precursor cell receptor for OPG was similarly cloned by screening an expression library with a labeled OPG. The receptor had many of the features of a member of the tumor

necrosis factor receptor family and appears to the murine equiv. of the human RANK protein. The external domain of the receptor was manufd. as a fusion protein with human IgG1 Fc and shown to inhibit bone resorption in mice as measured at the proximal tibial growth plate.

L8 ANSWER 34 OF 55 BIOSIS COPYRIGHT 2000 BIOSIS
1999:71302 Document No.: PREV199900071302. **Osteoprotegerin binding proteins.** Boyle, W. J.. Moorpark, Calif. USA. ASSIGNEE: AMGEN INC.. Patent Info.: US 5843678 Dec. 1, 1998. Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 1, 1998) Vol. 1217, No. 1, pp. 472. ISSN: 0098-1133. Language: English.

L8 ANSWER 35 OF 55 MEDLINE DUPLICATE 22
1998438470 Document Number: 98438470. Transforming growth factor-beta stimulates the production of osteoprotegerin/osteoclastogenesis inhibitory factor by bone marrow stromal cells. Takai H; Kanematsu M; Yano K; Tsuda E; Higashio K; Ikeda K; Watanabe K; Yamada Y. (Department of Geriatric Research, National Institute for Longevity Sciences, Obu, Aichi 474-8522, Japan.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Oct 16) 273 (42) 27091-6. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB **Osteoprotegerin** (**OPG**)/osteoclastogenesis inhibitory factor (OCIF) is a recently identified cytokine that belongs to the tumor necrosis factor **receptor** superfamily and regulates bone mass by inhibiting osteoclastic bone resorption. The present study was undertaken to determine whether OPG/OCIF is produced in bone microenvironment and how the expression is regulated. A transcript for OPG/OCIF at 3.1 kilobases was detected in bone marrow stromal cells (ST2 and MC3T3-G2/PA6) as well as in osteoblastic cells (MC3T3-E1). Transforming growth factor-beta1 (TGF-beta1) markedly increased the steady-state level of OPG/OCIF mRNA in a dose-dependent manner, while TGF-beta1 suppressed the mRNA expression of tumor necrosis factor-related activation-induced cytokine (TRANCE)/receptor activator of NF-kappaB ligand (RANKL), a positive regulator of osteoclastogenesis to which OPG/OCIF binds. The effect of TGF-beta1 on the expression of OPG/OCIF mRNA was transient, with a peak level at 3-6 h. The up-regulation of OPG/OCIF mRNA by TGF-beta1 in ST2 cells did not require de novo protein synthesis and involved both a transcriptional and a post-transcriptional mechanism. Western blot analysis and an enzyme-linked immunosorbent assay revealed that TGF-beta1 significantly increased the secretion of OPG/OCIF protein by ST2 cells at 6-24 h. In murine bone marrow cultures, TGF-beta1 markedly inhibited the formation of tartrate-resistant acid phosphatase-positive multinucleated osteoclast-like cells in the presence of 1,25-dihydroxyvitamin D3, whose effect was significantly reversed by a neutralizing antibody against OPG/OCIF. These results suggest that TGF-beta1 negatively regulates osteoclastogenesis, at least in part, through the induction of OPG/OCIF by bone marrow stromal cells and that the balance between OPG/OCIF and TRANCE/RANKL in local environment may be an important determinant of osteoclastic bone resorption.

L8 ANSWER 36 OF 55 MEDLINE DUPLICATE 23
1998269100 Document Number: 98269100. **Osteoprotegerin** is a **receptor** for the cytotoxic ligand TRAIL. Emery J G; McDonnell P; Burke M B; Deen K C; Lyn S; Silverman C; Dul E; Appelbaum E R; Eichman C; DiPrinzio R; Dodds R A; James I E; Rosenberg M; Lee J C; Young P R.

(Department of Molecular Biology, King of Prussia, Pennsylvania 19406, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Jun 5) 273 (23) 14363-7. Journal code: HIV. ISSN: 0021-9258. Pub. country: United States.
Language:
English.

AB TRAIL is a tumor necrosis factor-related ligand that induces apoptosis upon binding to its death domain-containing receptors, DR4 and DR5. Two additional TRAIL receptors, TRID/DcR1 and DcR2, lack functional death domains and function as decoy receptors for TRAIL. We have identified a fifth TRAIL receptor, namely **osteoprotegerin** (**OPG**), a secreted tumor necrosis factor **receptor** homologue that inhibits osteoclastogenesis and increases bone density in vivo. OPG-Fc binds TRAIL with an affinity of 3.0 nM, which is slightly weaker than the interaction of TRID-Fc or DR5-Fc with TRAIL. OPG inhibits TRAIL-induced apoptosis of Jurkat cells. Conversely, TRAIL blocks the anti-osteoclastogenic activity of OPG. These data suggest potential cross-regulatory mechanisms by OPG and TRAIL.

L8 ANSWER 37 OF 55 MEDLINE DUPLICATE 24

1999049841 Document Number: 99049841. **OPG**/FDCR-1, a TNF **receptor** family member, is expressed in lymphoid cells and is up-regulated by ligating CD40. Yun T J; Chaudhary P M; Shu G L; Frazer J K; Ewings M K; Schwartz S M; Pascual V; Hood L E; Clark E A. (Department of Immunology, University of Washington, Seattle 98195, USA.) JOURNAL OF IMMUNOLOGY, (1998 Dec 1) 161 (11) 6113-21. Journal code: IFB. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB We have cloned a TNFR family member from a follicular dendritic cell (FDC)-like cell line, FDC-1. This molecule, FDC-derived receptor-1 (FDCR-1), is identical to **osteoprotegerin** (**OPG**), a soluble cytokine that regulates osteoclast differentiation. Recently, **OPG**/FDCR-1 has been characterized as a second **receptor** for **receptor** activator of NF-kappaB ligand (RANKL)/TNF-related activation-induced cytokine (TRANCE), a primarily T-cell restricted TNF family member that augments dendritic cell (DC) function. In this report, we demonstrate that OPG/FDCR-1 is membrane bound on the surface of transfected baby hamster kidney (BHK) and untransfected FDC-1 cells. We also found a restricted OPG/FDCR-1 expression pattern in lymphoid cells, specifically in B cells, DCs and FDC-enriched fractions, which in B cells and DCs is up-regulated by CD40 stimulation. Because **OPG**/FDCR-1 shares some properties with RANK, the first RANKL/TRANCE **receptor**, we discuss how the balance between RANK and OPG/FDCR-1 expression could influence immune responses and, ultimately, germinal center formation.

L8 ANSWER 38 OF 55 MEDLINE DUPLICATE 25

1998389452 Document Number: 98389452. Hypocalcemic effect of osteoclastogenesis inhibitory factor/osteoprotegerin in the thyroparathyroidectomized rat. Yamamoto M; Murakami T; Nishikawa M; Tsuda E; Mochizuki S; Higashio K; Akatsu T; Motoyoshi K; Nagata N. (Third Department of Internal Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan.) ENDOCRINOLOGY, (1998 Sep) 139 (9) 4012-5. Journal code: EGZ. ISSN: 0013-7227. Pub. country: United States.
Language:
English.

AB Osteoclastogenesis inhibitory factor (OCIF), also termed as **osteoprotegerin** (**OPG**), is a soluble member of the tumor necrosis factor **receptor** family. Although OCIF/OPG is shown to inhibit osteoclast formation in vitro and prevent ovariectomy-induced bone loss in vivo, its effect on serum calcium level remains to be determined. In this study we examined the acute effect of OCIF on thyroparathyroidectomized rats whose serum calcium concentrations were

raised either by exogenous PTH or 1,25-(OH)2D3. When OCIF was administered at the start of PTH infusion, it attenuated the initial rise in serum calcium. When OCIF was administered into rats with established hypercalcemia, it decreased serum calcium rapidly (within 2 hr) and dramatically. OCIF did not increase urinary calcium excretion. These findings, especially the rapid onset of its hypocalcemic effect, suggest that OCIF not only inhibits the formation of osteoclasts but also affects the function and/or survival of mature osteoclasts at doses used in this study.

L8 ANSWER 39 OF 55 MEDLINE DUPLICATE 26
1998188248 Document Number: 98188248. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Yasuda H; Shima N; Nakagawa N; Yamaguchi K; Kinosaki M; Mochizuki S; Tomoyasu A; Yano K; Goto M; Murakami A; Tsuda E; Morinaga T; Higashio K; Udagawa N; Takahashi N; Suda T. (Research Institute of Life Science, Snow Brand Milk Products Co., Ltd., 519 Ishibashi-machi, Shimotsuga-gun, Tochigi 329-0512, Japan.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Mar 31) 95 (7) 3597-602. Journal code: PV3. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Osteoclasts, the multinucleated cells that resorb bone, develop from hematopoietic cells of monocyte/macrophage lineage. Osteoclast-like cells (OCLs) are formed by coculturing spleen cells with osteoblasts or bone marrow stromal cells in the presence of bone-resorbing factors. The cell-to-cell interaction between osteoblasts/stromal cells and osteoclast progenitors is essential for OCL formation. Recently, we purified and molecularly cloned osteoclastogenesis-inhibitory factor (OCIF), which was identical to **osteoprotegerin (OPG)**. **OPG**/OCIF is a secreted member of the tumor necrosis factor **receptor** family and inhibits osteoclastogenesis by interrupting the cell-to-cell interaction. Here we report the expression cloning of a ligand for OPG/OCIF from a complementary DNA library of mouse stromal cells. The protein was found to be a member of the membrane-associated tumor necrosis factor ligand family and induced OCL formation from osteoclast progenitors. A genetically engineered soluble form containing the extracellular domain of the protein induced OCL formation from spleen cells in the absence of osteoblasts/stromal cells. OPG/OCIF abolished the OCL formation induced by the protein. Expression of its gene in osteoblasts/stromal cells was up-regulated by bone-resorbing factors. We conclude that the membrane-bound protein is osteoclast differentiation factor (ODF), a long-sought ligand mediating an essential signal to osteoclast progenitors for their differentiation into osteoclasts. ODF was found to be identical to TRANCE/RANKL, which enhances T-cell growth and dendritic-cell function. ODF seems to be an important regulator in not only osteoclastogenesis but also immune system.

L8 ANSWER 40 OF 55 MEDLINE DUPLICATE 27
1998151033 Document Number: 98151033. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro. Yasuda H; Shima N; Nakagawa N; Mochizuki S I; Yano K; Fujise N; Sato Y; Goto M; Yamaguchi K; Kuriyama M; Kanno T; Murakami A; Tsuda E; Morinaga T; Higashio K. (Research Institute of Life Science, Snow Brand Milk Products Co., Ltd., Tochigi, Japan.. fvbd7042@mb.infoweb.or.jp) . ENDOCRINOLOGY, (1998 Mar) 139 (3)

1329-37. Journal code: EGZ. ISSN: 0013-7227. Pub. country: United States.
Language: English.
AB The morphogenesis and remodeling of bone depends on the integrated activity of osteoblasts that form bone and osteoclasts that resorb bone. We previously reported the isolation of a new cytokine termed osteoclastogenesis inhibitory factor, OCIF, which specifically inhibits osteoclast development. Here we report the cloning of a complementary DNA of human OCIF. OCIF is identical to **osteoprotegerin** (**OPG**), a soluble member of the tumor-necrosis factor **receptor** family that inhibits osteoclastogenesis. Recombinant human OPG/OCIF specifically acts on bone tissues and increases bone mineral density and bone volume associated with a decrease of active osteoclast number in normal rats. Osteoblasts or bone marrow-derived stromal cells support osteoclastogenesis through cell-to-cell interactions. A single class of high affinity binding sites for OPG/OCIF appears on a mouse stromal cell line, ST2, in response to 1,25-dihydroxyvitamin D3. An anti-OPG/OCIF antibody that blocks the binding abolishes the biological activity of OPG/OCIF. When the sites are blocked with OPG/OCIF, ST2 cells fail to support osteoclastogenesis. These results suggest that the sites are involved in cell-to-cell signaling between stromal cells and osteoclast progenitors and that OPG/OCIF inhibits osteoclastogenesis by interrupting the signaling through the sites.

L8 ANSWER 41 OF 55 MEDLINE DUPLICATE 28
1999003534 Document Number: 99003534. Osteoprotegerin production by human osteoblast lineage cells is stimulated by vitamin D, bone morphogenetic protein-2, and cytokines. Hofbauer L C; Dunstan C R; Spelsberg T C; Riggs B L; Khosla S. (Endocrine Research Unit, Mayo Clinic and Mayo Foundation, Rochester, Minnesota, USA.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Sep 29) 250 (3) 776-81. Journal code: 9Y8. ISSN: 0006-291X. Pub. country: United States. Language: English.
AB **Osteoprotegerin** (**OPG**), a newly discovered member of the tumor necrosis factor **receptor** family, is a potent inhibitor of osteoclastogenesis. The overexpression of OPG in transgenic mice leads to osteopetrosis, whereas targeted ablation of OPG in knock-out mice leads to severe osteoporosis. However, the production and regulation of OPG in normal human bone has not been studied. Thus, we assessed OPG mRNA expression and protein secretion in human osteoblastic lineage cells. 1,25-Dihydroxyvitamin D3 (10(-7) M) increased OPG mRNA levels by 90 and 50% in a fetal osteoblastic cell line (hFOB) and normal trabecular osteoblastic cells (hOB) cells, respectively, but did not affect OPG mRNA levels in a marrow stromal preosteoblastic (hMS) cell line. Interleukin (IL)-1beta (5 x 10(-9) M), tumor necrosis factor (TNF)-alpha (9 x 10(-9) M), and bone morphogenetic protein (BMP)-2 (100 ng/ml) also increased OPG mRNA levels in hFOB cells by 4-, 6-, and 4-fold, respectively. Treatment with 1,25-dihydroxyvitamin D3, IL-1beta, TNF-alpha, and BMP-2 increased OPG protein production by hFOB cells by 60, 390, 300, and 80%, respectively ($P < 0.001$). Because it is expressed in various types of human osteoblastic cells, and is stimulated by vitamin D, BMP-2 and cytokines, OPG may be an important paracrine modulator of bone remodeling.

L8 ANSWER 42 OF 55 MEDLINE DUPLICATE 29
1999057573 Document Number: 99057573. Transforming growth factor-betal increases mRNA levels of osteoclastogenesis inhibitory factor in osteoblastic/stromal cells and inhibits the survival of murine osteoclast-like cells. Murakami T; Yamamoto M; Ono K; Nishikawa M; Nagata N; Motoyoshi K; Akatsu T. (Third Department of Internal Medicine, Second

Department of Biochemistry, National Defense Medical College, Namiki 3-2, Saitama, Tokorozawa, 359-8513, Japan.. takkunn@ndmc.ac.jp) . BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Nov 27) 252 (3) 747-52. Journal code: 9Y8. ISSN: 0006-291X. Pub. country: United States.

Language:
English.

AB Osteoclastogenesis inhibitory factor (OCIF), also termed **osteoprotegerin** (**OPG**), is a secreted member of the tumor necrosis factor (TNF) **receptor** family. It inhibits bone resorption in vivo and osteoclast-like cell (OCL) formation in vitro. To better understand the biological role of OCIF, we first examined the effects of various osteotropic agents on OCIF mRNA levels in mouse calvarial osteoblasts. Northern blot analysis showed that stimulators of OCL formation such as 1,25-(OH)2D3, prostaglandin E2 (PGE2), parathyroid hormone (PTH), and interleukin 1 (IL-1) decreased OCIF mRNA levels. In contrast, transforming growth factor (TGF)-beta1 increased OCIF mRNA levels in primary osteoblasts as well as in osteoblastic/stromal cell lines. Since it was reported that both TGF-beta1 and OCIF not only inhibited OCL formation but also impaired the survival of OCL by inducing apoptosis in vitro, we next examined the possible involvement of OCIF in TGF-beta1-induced impairment of OCL survival. In a mouse bone marrow culture, we confirmed that addition of OCIF or TGF-beta1 decreased the number of surviving OCL. Anti-OCIF IgG, which completely neutralized the effect of OCIF, partially prevented the TGF-beta1-induced decrease in the number of OCL. Our results suggest that (i) downregulation of OCIF expression is one of the mechanisms for the stimulatory effects of 1,25(OH)2D3, PGE2, PTH, and IL-1 on osteoclastogenesis; and (ii) the TGF-beta1-induced apoptosis of OCL is mediated, at least in part, by upregulation of OCIF expression. Copyright 1998 Academic Press.

L8 ANSWER 43 OF 55 CAPLUS COPYRIGHT 2000 ACS
1999:24510 Document No. 130:208170 Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. Pitti, Robert M.; Marsters, Scot A.; Lawrence, David A.; Roy, Margaret; Kischkel, Frank C.; Dowd, Patrick; Huang, Arthur; Donahue, Christopher J.; Sherwood, Steven W.; Baldwin, Daryl T.; Godowski, Paul J.; Wood, William I.; Gurney, Austin L.; Hillan, Kenneth J.; Cohen, Robert L.; Goddard, Audrey D.; Botstein, David; Ashkenazi, Avi (Departments of Molecular Oncology, Molecular Biology, and Immunology, Genentech Inc., San Francisco, CA, 94080, USA). Nature (London), 396(6712), 699-703 (English) 1998. CODEN: NATUAS. ISSN: 0028-0836. Publisher: Macmillan Magazines.

AB Fas ligand (FasL) is produced by activated T cells and natural killer cells and it induces apoptosis (programmed cell death) in target cells through the death receptor Fas/Apo1/CD95. One important role of FasL and Fas is to mediate immune-cytotoxic killing of cells that are potentially harmful to the organism, such as virus-infected or tumor cells. Here the authors report the discovery of a sol. decoy receptor, termed decoy receptor 3 (DcR3), that binds to FasL and inhibits FasL-induced apoptosis.

The DcR3 gene was amplified in about half of 35 primary lung and colon tumors studied, and DcR3 mRNA was expressed in malignant tissue. Thus, certain tumors may escape FasL-dependent immune-cytotoxic attack by expressing a decoy receptor that blocks FasL.

L8 ANSWER 44 OF 55 MEDLINE DUPLICATE 30
1998369612 Document Number: 98369612. Osteoprotegerin mRNA is increased by interleukin-1 alpha in the human osteosarcoma cell line MG-63 and in human osteoblast-like cells. Vidal O N; Sjogren K; Eriksson B I; Ljunggren O;

Ohlsson C. (Endocrine Bone Unit, Sahlgrenska University Hospital, Gothenburg, Sweden.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Jul 30) 248 (3) 696-700. Journal code: 9Y8. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB **Osteoprotegerin** (**OPG**) is a soluble **receptor** for the Osteoprotegerin-Ligand (OPGL) which is expressed on osteoblasts and mediates the signal for osteoclast differentiation. In the present study we demonstrate that OPG mRNA levels in MG-63 cells are increased in a dose-dependent manner after 8 h of treatment with IL-1 alpha (338 +/- 53% over control at 25 U/ml). Interleukin-6 (IL-6), under similar culture conditions, does not affect OPG mRNA levels. Time-course studies show that IL-1 alpha (25 U/ml) causes a transient increase of OPG mRNA levels in MG-63 cells, peaking after 4 h of treatment. An increase of the OPG transcript occurs in hOB cells at 0.5 h which is still present after 24 h of IL-1 alpha treatment. In MG-63 cells neither basal-nor IL-1 alpha-induced OPG mRNA levels are altered by the translational inhibitor cycloheximide. These results suggest that expression of OPG in osteoblasts may be regulated by IL-1 alpha.

L8 ANSWER 45 OF 55 MEDLINE DUPLICATE 31

1998321175 Document Number: 98321175. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. Mizuno A; Amizuka N; Irie K; Murakami A; Fujise N; Kanno T; Sato Y; Nakagawa N; Yasuda H; Mochizuki S; Gomibuchi T; Yano K; Shima N; Washida N; Tsuda E; Morinaga T; Higashio K; Ozawa H. (Research Institute of Life Science, Snow Brand Milk Products, Co., Ltd., Tochigi, Japan.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Jun 29) 247 (3) 610-5. Journal code: 9Y8. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB Osteoclasts are multinucleated cells that resorb bone. Osteoclastogenesis inhibitory factor (OCIF), also called **osteoprotegerin** (**OPG**), acts as a naturally occurring decoy **receptor** for osteoclast differentiation factor, which mediates an essential signal to osteoclast progenitors for their differentiation into osteoclasts. Here we show that the OCIF/OPG knockout mice exhibited severe osteoporosis due to enhanced osteoclastogenesis when they grew to be adults. These mice were viable and fertile. They exhibited marked bone loss accompanied by destruction of growth plate and lack of trabecular bone in their femurs. The strength of their bones dramatically decreased. These results demonstrate that OCIF/OPG is a key factor acting as a negative regulator against osteoclastogenesis. The OCIF/OPG knockout mice provide the first animal model for osteoporosis without other obvious abnormalities.

L8 ANSWER 46 OF 55 MEDLINE DUPLICATE 32

1998369568 Document Number: 98369568. Tumor necrosis factor-alpha and -beta upregulate the levels of osteoprotegerin mRNA in human osteosarcoma MG-63 cells. Brandstrom H; Jonsson K B; Vidal O; Ljunghall S; Ohlsson C; Ljunggren O. (Department of Medical Sciences, University of Uppsala, Sweden.. Helena.Brandstrom@medicin.uu.se) . BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Jul 30) 248 (3) 454-7. Journal code: 9Y8. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB **Osteoprotegerin** (**OPG**) is a recently cloned soluble member of the tumor necrosis factor **receptor** family. OPG has been shown to inhibit osteoclast recruitment by binding to OPG-ligand, an osteoclast differentiating factor on osteoblastic stromal cells, thereby blocking osteoclastogenesis. In this report we have examined the effect of

tumor necrosis factor-alpha (TNF-alpha) and tumor necrosis factor-beta (TNF-beta) on OPG mRNA levels in the human osteosarcoma cell line MG-63. We demonstrate that both TNF-alpha and TNF-beta dose- and time-dependently upregulate the mRNA levels of OPG. The effect is significant at and above 5 pM of TNF-alpha and 1 pM of TNF-beta. The stimulatory effect on OPG mRNA levels in MG-63 cells was detected after 2 hrs of incubation with TNF-alpha or TNF-beta. These data demonstrate that the expression of OPG in osteoblasts, with subsequent effects on osteoclastogenesis, is regulated by TNFs.

L8 ANSWER 47 OF 55 MEDLINE DUPLICATE 33

1999069519 Document Number: 99069519. Osteoprotegerin mRNA is expressed in primary human osteoblast-like cells: down-regulation by glucocorticoids. Vidal N O; Brandstrom H; Jonsson K B; Ohlsson C. (Research Centre for Endocrinology and Metabolism, Department of Internal Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden.) JOURNAL OF ENDOCRINOLOGY, (1998 Oct) 159 (1) 191-5. Journal code: I1J. ISSN: 0022-0795. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Osteoprotegerin** (**OPG**) is a recently cloned member of the tumour necrosis factor **receptor** family. It has been suggested that this secreted glycoprotein acts as an inhibitor of osteoclastic differentiation. Expression of OPG has previously been demonstrated in a number of tissues. However, it is still unclear whether or not OPG is expressed by human osteoblasts. We have used the RNase protection assay to demonstrate the OPG transcript in primary cultured human osteoblast-like cells, human marrow stroma cells and osteosarcoma cell lines. Furthermore, we have studied the effect of glucocorticoids on OPG mRNA levels in these cells. We demonstrate that glucocorticoids decrease the OPG transcript in a dose- and time-dependent manner. The time-course study reveals that hydrocortisone (10(-6) M) decreases OPG mRNA levels within 2 h. This decrease is transient, reaching control levels again after 24 h. Our findings demonstrate that human osteoblasts express the mRNA corresponding to OPG, an inhibitor of osteoclast differentiation. The finding that OPG mRNA levels are decreased by glucocorticoids indicates that a reduced production of OPG from osteoblasts and/or marrow stroma cells could, in part, explain glucocorticoid-induced bone resorption.

L8 ANSWER 48 OF 55 CAPLUS COPYRIGHT 2000 ACS

1998:163869 Document No. 128:176217 Overview. Watanabe, Ken; Ikeda, Kyoji (Dep. Geriatr. Res., Natl. Inst. Longevity Sci., Obu, 474, Japan). Horumon to Rinsho, 46(3), 167-175 (Japanese) 1998. CODEN: HORIAE. ISSN: 0045-7167. Publisher: Igaku no Sekai Sha.

AB A review with 60 refs. on expression and function of **osteoprotegerin**, involvement of PTH-related peptide, Indian hedgehog, fibroblast growth factor **receptor**, etc., in endochondral bone formation, and significance of bone morphogenetic proteins in signal transduction.

L8 ANSWER 49 OF 55 CAPLUS COPYRIGHT 2000 ACS

1998:549094 Document No. 129:274334 Osteoprotegerin and its cognate ligand: a new paradigm of osteoclastogenesis. Hofbauer, Lorenz C.; Heufelder, Armin E. (Endocrine Research Unit, Mayo Clinic, Rochester, MN, 55905, USA). Eur. J. Endocrinol., 139(2), 152-154 (English) 1998. CODEN: EJOEEP. ISSN: 0804-4643. Publisher: BioScientifica.

AB A review and discussion with 11 refs. **Osteoprotegerin** (**OPG**) is a member of the tumor necrosis factor **receptor**

superfamily. OPG and its cognate ligand (OPGL) are a cybernetic couple that regulate bone mass by modulating osteoclastogenesis. OPGL seems to be the endogenous master cytokine, which is the condition sine qua non for normal osteoclast differentiation and activation, whereas **OPG** is a naturally occurring sol. **receptor** that counterbalances the effects of OPGL and preserves bone mass. Mechanisms of action of OPG and OPGL are presented.

L8 ANSWER 50 OF 55 CAPLUS COPYRIGHT 2000 ACS
1998:68727 Document No. 128:113208 Osteoprotegerin. A novel secreted osteoclastogenesis inhibitor. Boyle, William J. (Dep. Cell Biol, Amgen, Inc., Thousand Oaks, 91320-1789, USA). Jikken Igaku, 16(2), 137-141 (Japanese) 1998. CODEN: JIIGEF. ISSN: 0288-5514. Publisher: Yodosha.
AB A review with 11 refs. Osteoprotegerin (OPG) is a novel member of the TNFR superfamily that acts as a neg. regulator of osteoclast development. This implies that the terminal stages of osteoclast maturation are under the control of a TNF related protein that acts as a osteoclast differentiation factor. **OPG** is therefore a sol. decoy **receptor** that interferes with the action of the osteoclast differentiation factor, and blocks pre-osteoclast differentiation. The resulting effect in vivo is to block bone resorption, leading to the accumulation of bone mass. OPG may prove to be a key determinant in the regulation of bone mass, and further study of this novel protein may provide insight into the humoral regulation of bone d. The mol. action of OPG is not known. However, it is seems likely that it will provide great insight into the mechanisms involved in the regulating osteoclast differentiation from hematopoietic precursors. Since the osteoclast plays a central role in osteopenic disease processes, the anal. of OPG is also likely to provide an insight into the pathol. mechanisms underlying osteoporosis.

L8 ANSWER 51 OF 55 MEDLINE DUPLICATE 34
1998380602 Document Number: 98380602. Interleukins in the control of osteoclast differentiation. Martin T J; Romas E; Gillespie M T. (St. Vincent's Institute of Medical Research, Victoria, Australia.) CRITICAL REVIEWS IN EUKARYOTIC GENE EXPRESSION, (1998) 8 (2) 107-23. Ref: 143. Journal code: BEJ. ISSN: 1045-4403. Pub. country: United States.
Language:
English.
AB To maintain homeostasis of bone, the production of osteoblasts and osteoclasts is tightly regulated. At the local level, hormones and cytokines control formation of osteoclasts from hemopoietic precursors by acting upon osteoblastic-stromal cells and in some cases also upon cells of the immune system. Osteoblasts regulate osteoclast formation by providing physical support and cytokines such as M-CSF and IL-11, which promote osteoclast differentiation. Osteoblasts are also a source of IL-18, which limits osteoclast formation. The requirement of contact between osteoblasts and hemopoietic cells for successful osteoclast formation led to a concept of a membrane-anchored stromal cell molecule that programs osteoclast differentiation. This mechanism has been highlighted by the discovery of osteoprotegerin (OPG), a soluble tumor necrosis factor (TNF) family member that inhibits osteoclast formation. The ligand for **OPG** is a novel transmembrane TNF **receptor** superfamily member, the osteoclast differentiating factor (ODF). The recognition of the osteoprotegerin/osteoprotegerin-ligand axis will lead to new insights into the control of osteoclast differentiation by interleukins.

L8 ANSWER 52 OF 55 BIOSIS COPYRIGHT 2000 BIOSIS
1998:458038 Document No.: PREV199800458038. **Osteoprotegerin** is a **receptor** for the cytotoxic ligand trail. Emery, J. G.; McDonnell, P.; Burke, M. Brigham; Deen, K. C.; Lyn, S. D.; Silverman, Carol; Dul, E.; Appelbaum, E. R.; Eichman, C.; Diprinzio, R.; Dodds, R. A.; James, I. E.; Rosenberg, M.; Lee, J. C.; Young, P. R.. Dep. Molecular Biol., SmithKline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, PA 19406 USA. Journal of Interferon and Cytokine Research, (May, 1998) Vol. 18, No. 5, pp. A47. Meeting Info.: 7th International Conference on Tumor Necrosis Factor and Related Molecules Scientific Advances and Medical Applications Hyannis, Massachusetts, USA May 17-21, 1998 ISSN: 1079-9907. Language: English.

L8 ANSWER 53 OF 55 CAPLUS COPYRIGHT 2000 ACS
1997:496801 Document No. 127:131591 **Osteoprotegerin**: a member of the TNF **receptor** superfamily involved in the regulation of bone density and a cDNA encoding it. Boyle, William J.; Lacey, David L.; Calzone, Frank J.; Chang, Ming-shi (Amgen Inc., USA). Ger. Offen. DE 19654610 A1 19970626, 189 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1996-19654610 19961220. PRIORITY: US 1995-577788 19951222; US 1996-706945 19960903.
AB A new secreted glycoprotein that is a member of the tumor necrosis factor receptor superfamily, dubbed osteoprotegerin, is identified and rat, mouse, and human cDNAs encoding it are cloned. The protein is involved in the regulation of bone d. and may be of therapeutic use in the treatment of bone diseases such as resorption and osteoporosis (no data). Cloning and expression of the gene in transgenic mice is reported. Antibodies to the protein and pharmaceutically useful forms of the protein are described. The cDNA was first identified as an expressed sequence tag in a fetal rat intestine cDNA library. Tissue distribution studies showed the mRNA to be widely distributed with some tissues showing a 2.4 kb transcript and others showing a 4.5-7.5 kb transcript. Expression of the cDNA from the ApoE promoter in transgenic mice did not have have any adverse effects on most organs except for the spleen, which was enlarged. Necropsies showed very heavy osteopetrosis. Application of osteoprotegerins to sites treated with interleukin 1.alpha. or 1.beta. to induce bone resorption inhibited the resorption.

L8 ANSWER 54 OF 55 MEDLINE DUPLICATE 35
97262071 Document Number: 97262071. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density [see comments]. Simonet W S; Lacey D L; Dunstan C R; Kelley M; Chang M S; Luthy R; Nguyen H Q; Wooden S; Bennett L; Boone T; Shimamoto G; DeRose M; Elliott R; Colombero A; Tan H L; Trail G; Sullivan J; Davy E; Bucay N; Renshaw-Gegg L; Hughes T M; Hill D; Pattison W; Campbell P; Boyle W J; et al. (Department of Molecular Genetics, Amgen Inc., Thousand Oaks, California 91320, USA.) CELL, (1997 Apr 18) 89 (2) 309-19. Journal code: CQ4. ISSN: 0092-8674. Pub. country: United States. Language: English.
AB A novel secreted glycoprotein that regulates bone resorption has been identified. The protein, termed **Osteoprotegerin (OPG)**, is a novel member of the TNF **receptor** superfamily. In vivo, hepatic expression of OPG in transgenic mice results in a profound yet nonlethal osteopetrosis, coincident with a decrease in later stages of osteoclast differentiation. These same effects are observed upon administration of recombinant OPG into normal mice. In vitro, osteoclast differentiation from precursor cells is blocked in a dose-dependent manner

by recombinant OPG. Furthermore, OPG blocks ovariectomy-associated bone loss in rats. These data show that OPG can act as a soluble factor in the regulation of bone mass and imply a utility for OPG in the treatment of osteoporosis associated with increased osteoclast activity.

L8 ANSWER 55 OF 55 CAPLUS COPYRIGHT 2000 ACS
1998:29120 Document No. 128:165342 Characterization of a novel TNF-like ligand and recently described TNF ligand and TNF receptor superfamily genes and their constitutive and inducible expression in hematopoietic and non-hematopoietic cells. Tan, K. B.; Harrop, Jeremy; Reddy, Manjula; Young, Peter; Terrett, Jonathan; Emery, John; Moore, Gordon; Truneh, Alemseged (Department Molecular Immunology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA, 19406-0939, USA). Gene, 204(1/2), 35-46 (English) 1997. CODEN: GENED6. ISSN: 0378-1119. Publisher: Elsevier Science B.V..

AB A novel (TL1), a recently described (TL2) TNF-like, and three recently described TNF receptor-like (TR1, TR2, TR3) mols. were identified by searching a cDNA database. TL1 and TL2 are type-II membrane proteins. TR2 and TR3 and type-I membrane proteins whereas TR1 appears to be a secreted protein. TL1, TL2, TR2 and TR3 were expressed in hematopoietic cells, whereas TR1 was not. Northern blots hybridized with the cDNA probes revealed multiple forms of RNA as well as inducible expression of TL1, TL2, TR2 and TR3. TL2 and TR3, in particular, were highly induced in activated CD4+ T cells. Radiation hybrid mapping localized TR1 and TL2 to 8q24 and 3q26, resp., which are not near any known superfamily members. TL1 was mapped to 9q32, near CD30L (9q33) and TR2 and TR3 mapped to the region of chromosome 1 that contains the TNFR-II, 4-1BB, OX40 and CD30 gene cluster at 1p36. Only TR3 in this cluster possesses a death domain. Southern blot anal. revealed the presence of TL and TR genes in different mammalian species. TL2, TR1, TR2 and TR3 were recently described by others as TRAIL/Apo-2L, OPG, HVEM and DR3/WSL-1/Apo-3/TRAMP/LARD, resp.

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L9          11 FILE MEDLINE
L10         14 FILE CAPLUS
L11          7 FILE BIOSIS
L12         16 FILE EMBASE
L13          2 FILE WPIDS

TOTAL FOR ALL FILES
L14         50 (L1 OR PSTEOPTOTEGERIN OR OPG) AND ANTIBOD?

=> s l14 not l7

L15          5 FILE MEDLINE
L16          6 FILE CAPLUS
L17          3 FILE BIOSIS
L18         10 FILE EMBASE
L19          1 FILE WPIDS

TOTAL FOR ALL FILES
L20         25 L14 NOT L7

=> dup rem l20

PROCESSING COMPLETED FOR L20
L21          17 DUP REM L20 (8 DUPLICATES REMOVED)
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=> d cbib abs 1-17

L21 ANSWER 1 OF 17 MEDLINE
2000155531 Document Number: 20155531. Involvement of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Takayanagi H; Iizuka H; Juji T; Nakagawa T; Yamamoto A; Miyazaki T; Koshihara Y; Oda H; Nakamura K; Tanaka S. (The University of Tokyo, Japan.) ARTHRITIS AND RHEUMATISM, (2000 Feb) 43 (2) 259-69. Journal code: 90M. ISSN: 0004-3591.
Pub. country: United States. Language: English.
AB OBJECTIVE: To clarify the mechanism by which osteoclasts are formed in culture of rheumatoid synoviocytes by exploring the involvement of receptor activator of nuclear factor kappaB ligand (RANKL)/osteoclast differentiation factor (ODF). METHODS: Osteoclast formation was evaluated in cocultures of rheumatoid synovial fibroblasts and peripheral blood mononuclear cells (PBMC) in the presence of macrophage colony stimulating factor and 1,25-dihydroxyvitamin D3 (1,25[OH]2D3) utilizing separating membrane filters. RANKL/ODF expression was examined by Northern blotting in synovial tissues from 5 rheumatoid arthritis (RA) patients and tissues from patients with giant cell tumor (GCT), osteosarcoma (OS), and osteoarthritis (OA). RANKL/ODF expression and the ability of synovial fibroblasts to support osteoclastogenesis were investigated in coculture with PBMC in the presence or absence of 1,25(OH)2D3, and soluble RANKL/ODF and osteoprotegerin (**OPG**)/osteoclastogenesis inhibitory factor (OCIF) were measured by enzyme-linked immunosorbent assay. The effects of **OPG**/OCIF on the osteoclastogenesis in the primary culture of rheumatoid synoviocytes and the coculture system were determined. RESULTS: Synovial fibroblasts did not induce osteoclastogenesis when separately cocultured with PBMC. Northern blotting revealed that RANKL/ODF was highly expressed in all tissues from RA and GCT patients, but not from OA or OS patients. Cultured rheumatoid synovial fibroblasts efficiently induced osteoclastogenesis in the presence of 1,25(OH)2D3, which was accompanied by up-regulated expression of RANKL/ODF and decreased production of **OPG**/OCIF. Osteoclastogenesis from synoviocytes was dose-dependently inhibited by **OPG**/OCIF. CONCLUSION: RANKL/ODF expressed on synovial fibroblasts is involved in rheumatoid bone destruction by inducing osteoclastogenesis and would therefore be a good therapeutic target.

L21 ANSWER 2 OF 17 CAPLUS COPYRIGHT 2000 ACS
1999:795994 Document No. 132:31744 Gene probes used for genetic profiling in healthcare screening and planning. Roberts, Gareth Wyn (Genostic Pharma Ltd., UK). PCT Int. Appl. WO 9964627 A2 19991216, 745 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1780 19990604. PRIORITY: GB 1998-12099 19980606; GB 1998-13291 19980620; GB 1998-13611 19980624; GB 1998-13835 19980627; GB 1998-14110 19980701; GB 1998-14580 19980707; GB 1998-15438 19980716; GB

1998-15576 19980718; GB 1998-15574 19980718; GB 1998-16085 19980724; GB 1998-16086 19980724; GB 1998-16921 19980805; GB 1998-17097 19980807; GB 1998-17200 19980808; GB 1998-17632 19980814; GB 1998-17943 19980819.

AB There is considerable evidence that significant factor underlying the individual variability in response to disease, therapy and prognosis lies in a person's genetic make-up. There have been numerous examples relating that polymorphisms within a given gene can alter the functionality of the protein encoded by that gene thus leading to a variable physiol. response. In order to bring about the integration of genomics into medical practice and enable design and building of a technol. platform which will enable the everyday practice of mol. medicine a way must be invented for the DNA sequence data to be aligned with the identification of genes central to the induction, development, progression and outcome of disease or physiol. states of interest. According to the invention, the no. of genes and their configurations (mutations and polymorphisms) needed to be identified in order to provide crit. clin. information concerning individual prognosis is considerably less than the 100,000 thought to comprise the human genome. The identification of the identity of the core group of genes enables the invention of a design for genetic profiling technologies which comprises of the identification of the core group of genes and their sequence variants required to provide a broad base of clin. prognostic information - "genostics". The "Genostic.RTM." profiling of patients and persons will radically enhance the ability of clinicians, healthcare professionals and other parties to plan and manage healthcare provision and the targeting of appropriate healthcare resources to those deemed most in need. The use of this invention could also lead to a host of new applications for such profiling technologies, such as identification of persons with particular work or environment related risk, selection of applicants for employment, training or specific opportunities or for the enhancing of the planning and organization of health services, education services and social services.

L21 ANSWER 3 OF 17 CAPLUS COPYRIGHT 2000 ACS

1999:795993 Document No. 132:31743 Gene probes used for genetic profiling in healthcare screening and planning. Roberts, Gareth Wyn (Genostic Pharma Limited, UK). PCT Int. Appl. WO 9964626 A2 19991216, 149 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1779 19990604. PRIORITY: GB 1998-12098 19980606; GB 1998-28289 19981223.

AB There is considerable evidence that significant factor underlying the individual variability in response to disease, therapy and prognosis lies in a person's genetic make-up. There have been numerous examples relating that polymorphisms within a given gene can alter the functionality of the protein encoded by that gene thus leading to a variable physiol. response. In order to bring about the integration of genomics into medical practice

and enable design and building of a technol. platform which will enable the everyday practice of mol. medicine a way must be invented for the DNA sequence data to be aligned with the identification of genes central to the induction, development, progression and outcome of disease or physiol.
states of interest. According to the invention, the no. of genes and their configurations (mutations and polymorphisms) needed to be identified
in order to provide crit. clin. information concerning individual prognosis is considerably less than the 100,000 thought to comprise the human genome. The identification of the identity of the core group of genes enables the invention of a design for genetic profiling technologies.

L21 ANSWER 4 OF 17 CAPLUS COPYRIGHT 2000 ACS
1999:223074 Document No. 130:222129 Method for diagnosing bone dysbolism. Yano, Kazuki; Kobayashi, Fumie; Goto, Masaaki; Washida, Naohiro; Tsuda, Eisuke; Higashio, Kanji; Yamada, Yoshiji (Snow Brand Milk Products Co., Ltd., Japan). PCT Int. Appl. WO 9915691 A1 19990401, 36 pp. DESIGNATED STATES: W: AU, CA, CN, HU, IL, JP, KR, MX, NO, NZ, RU, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (Japanese). CODEN: PIXXD2. APPLICATION: WO 1998-JP3421 19980731. PRIORITY: JP 1997-276475 19970924.
AB A method for diagnosing bone dysbolism, in particular osteoporosis and joint diseases characterized by measuring the concn. of osteoclastogenesis
inhibitory factors (OCIFs) in the bodily fluid; a monoclonal **antibody** equally recognizing monomeric and dimeric OCIFs; a monoclonal **antibody** selectively recognizing the dimeric OCIF alone; and OCIF assay kits which contain the monoclonal **antibodies** of the above two types, recognizing different epitopes of OCIFs, and having a high affinity and a dissocn. const. with an antigen of 2 x 10-7 M
or below. Immunization of Balb/c mice by i.p. injection, collection of spleen of the immunized mice, hybridization with mouse myeloma P3x63-AG8.653, and growth of the monoclonal **antibody**-producing hybridoma by the ascite method were shown. The above **antibodies** and kits are useful in diagnosing bone dysbolism, in particular, osteoporosis and joint diseases or anal. reagents for lab. use, etc.

L21 ANSWER 5 OF 17 CAPLUS COPYRIGHT 2000 ACS
1998:434798 Document No. 129:184595 TR1, a new member of the tumor necrosis factor receptor superfamily, induces fibroblast proliferation and inhibits
osteoclastogenesis and bone resorption. Kwon, Byoung S.; Wang, S. A.; Udagawa, Nobuyuki; Haridas, Valsala; Lee, Zang H.; Kim, Kack K.; Oh, Kwi-Ok; Greene, John; Li, Yuling; Su, Jeffrey; Gentz, Reiner; Aggarwal, Bharat B.; Ni, Jian (Department of Microbiology and Immunology, Indiana University School of Medicine and the Walther Cancer Institute, Indianapolis, IN, 46202-5120, USA). FASEB J., 12(10), 845-854 (English) 1998. CODEN: FAJOEC. ISSN: 0892-6638. Publisher: Federation of American
Societies for Experimental Biology.
AB A newly identified member of the tumor necrosis factor receptor (TNFR) superfamily shows activities assocd. with osteoclastogenesis inhibition and fibroblast proliferation. This new member, called TR1, was identified
from a search of an expressed sequence tag database, and encodes 401 amino
acids with a 21-residue signal sequence. Unlike other members of TNFR, TR1 does not contain a transmembrane domain and is secreted as a 62 kDa

glycoprotein. TR1 gene maps to chromosome 8q23-24.1 and its mRNA is abundantly expressed on primary osteoblasts, osteogenic sarcoma cell lines, and primary fibroblasts. The receptors for TR1 were detected on a monocytic cell line (THP-1) and in human fibroblasts. Scatchard analyses indicated two classes of high and medium-high affinity receptors with a kD of approx. 45 and 320 pM, resp. Recombinant TR1 induced proliferation of human foreskin fibroblasts and potentiated TNF-induced proliferation in these cells. In a coculture system of osteoblasts and bone marrow cells, recombinant TR1 completely inhibited the differentiation of osteoclast-like multinucleated cell formation in the presence of several bone-resorbing factors. TR1 also strongly inhibited bone-resorbing function on dentin slices by mature osteoclasts and decreased 45Ca release in fetal long-bone organ cultures. Anti-TR1 monoclonal **antibody** promoted the formation of osteoclasts in mouse marrow culture assays. These results indicate that TR1 has broad biol. activities in fibroblast growth and in osteoclast differentiation and its functions.

L21 ANSWER 6 OF 17 MEDLINE DUPLICATE 1

1999097247 Document Number: 99097247. RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. Nakagawa N; Kinosaki M; Yamaguchi K; Shima N; Yasuda H; Yano K; Morinaga T; Higashio K. (Research Institute of Life Science, Snow Brand Milk Products Co., Ltd., Tochigi, Japan.. fvbd7042@mb.infoweb.ne.jp) . BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 Dec 18) 253 (2) 395-400. Journal code: 9Y8. ISSN: 0006-291X. Pub. country: United States.

Language: English.

AB Osteoclast differentiation factor (ODF) is a ligand for osteoclastogenesis-inhibitory factor/osteoprotegerin (OCIF/**OPG**), and mediates an essential signal for osteoclastogenesis. Soluble-form ODF binds directly to osteoclast progenitors, suggesting the presence of a membrane-bound receptor for ODF (ODFR) on the cells. To understand the ODF-mediated signal transduction mechanism in osteoclastogenesis, we molecularly cloned ODFR from a mouse macrophage-like osteoclast progenitor cell line, C7. Nucleotide sequence analysis revealed that ODFR is identical to RANK, a recently identified member of the tumor necrosis factor receptor (TNFR) family, which is involved in the regulation of dendritic cell function. A polyclonal **antibody** against the extracellular domain of RANK induced osteoclastogenesis in the presence of macrophage colony-stimulating factor (M-CSF). In contrast, both a genetically engineered soluble RANK and Fab fragment of the **antibody** blocked the binding of ODF to RANK and ODF-mediated osteoclastogenesis. These results indicate that RANK is the signaling receptor essential for ODF-mediated osteoclastogenesis. Copyright 1998 Academic Press.

L21 ANSWER 7 OF 17 MEDLINE DUPLICATE 2

1998273279 Document Number: 98273279. Osteoclast differentiation factor mediates an essential signal for bone resorption induced by 1 alpha,25-dihydroxyvitamin D3, prostaglandin E2, or parathyroid hormone in the microenvironment of bone. Tsukii K; Shima N; Mochizuki S; Yamaguchi K; Kinosaki M; Yano K; Shibata O; Udagawa N; Yasuda H; Suda T; Higashio K. (Research Institute of Life Science, Snow Brand Milk Products Co., Ltd., Tochigi, Japan.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1998 May 19) 246 (2) 337-41. Journal code: 9Y8. ISSN: 0006-291X. Pub.

country: United States. Language: English.

AB Osteoclast differentiation factor (ODF), a ligand for osteoprotegerin (**OPG**)/osteoclastogenesis-inhibitory factor (OCIF), induces osteoclast-like cell formation in vitro. To elucidate the role of ODF in the microenvironment of bone, we examined effects of ODF, **OPG** /OCIF, and anti-ODF polyclonal **antibody** on bone resorption using a fetal mouse long bone culture system. A genetically engineered soluble-form ODF (sODF) elicited bone resorption in a concentration-dependent manner and **OPG**/OCIF blocked the bone resorption. Anti-ODF polyclonal **antibody**, which neutralizes ODF activity, negated bone resorption induced by 1 alpha,25-dihydroxyvitamin D3, parathyroid hormone, or prostaglandin E2. **OPG**/OCIF also abolished bone-resorbing activity elicited by these bone-resorbing agents.

Interleukin 1 alpha-stimulated bone resorption was also significantly suppressed by anti-ODF polyclonal **antibody** and **OPG** /OCIF. Thus, we conclude that ODF plays a critical role in bone resorption in the microenvironment of bone.

L21 ANSWER 8 OF 17 WPIDS COPYRIGHT 2000 DERWENT INFORMATION LTD

AN 1997-334271 [31] WPIDS

AB DE 19654610 A UPAB: 19981021

New isolated nucleic acid (I), encoding a polypeptide (A) that has at least one of the biological activities of **OPG** (osteoprotegerin), is: (a) a 2432, 1324 or 1355 bp sequence, or their complements; (b) sequences that hybridise under stringent conditions with the polypeptide coding region of (a) (nts. 124-1329, 91-1296 and 95-1300 respectively); (c) a sequence that hybridises under stringent conditions with a fragment designated FRI-1 (nts. 148-373); (d) any sequence equivalent to (a)-(c) within the degeneracy of the genetic code. Also new are: (1) (A), including truncated forms, derivatives and modified forms; (2) related vectors, host cells, transgenic animals and **antibodies**.

USE - Ab are used for diagnostic assay of **OPG** and as affinity purification material. (I) is used to express recombinant **OPG** and to generate transgenic animals, also to regulate the level of **OPG** in mammals, specifically to increase the level but use of antisense sequences is also contemplated. Fragments of it are useful as probes to detect **OPG**-expressing cells and tissue, and for screening cDNA libraries for related sequences. (A) are used to treat or prevent bone diseases, specifically excessive bone loss such as (steroid-)induced osteoporosis, Paget's disease, hypercalcaemia (e.g. caused by solid tumours), hyperparathyroidism, rheumatoid arthritis, osteomyelitis, osteolytic metastases and periodontal bone loss, also (non)-traumatic bone necrosis and osteopaenia of various aetiologies. Dwg.28/28

L21 ANSWER 9 OF 17 MEDLINE DUPLICATE 3

96261127 Document Number: 96261127. Evaluation for detection of Cryptosporidium oocysts in diarrheal feces of calves. Wee S H; Joo H D; Kang Y B. (National Veterinary Research Institute, RDA, Anyang, Korea.) KOREAN JOURNAL OF PARASITOLOGY, (1996 Jun) 34 (2) 121-6. Journal code: B3D. ISSN: 0023-4001. Pub. country: KOREA. Language: English.

AB For the detection of Cryptosporidium oocysts, fecal samples were collected from 201 calves which showed diarrhea. Among the 201 samples, 29 samples (14.4%) were positive for Cryptosporidium spp. by the DMSO-modified acid-fast stain (MAFS), 23 samples (11.4%) were positive by commercial kit (Meridian Diagnostics, Cincinnati, Ohio) and 23 by the indirect immunofluorescence **antibody** (IFA) assay employing the monoclonal

antibody (mAb C6). When tested by both IFA and MAFS, 20 fecal samples were positive for Cryptosporidium oocysts whereas 169 fecal samples were negative. If the MAFS is considered a standard method for oocyst detection, the IFA showed 69% of sensitivity and 98% of specificity. When tested by both IFA and commercial kit, 22 fecal samples were positive for Cryptosporidium oocysts while 177 samples were negative.

One sample tested by IFA was found to be false negative, when compared with the results by commercial kit. The sensitivity of IFA was calculated as high as 96%; the specificity as 99% and the predictive value was also 99%. In the present study, IFA employing the mAb C6 revealed that 23 samples (11.4%) were positive among the 201 calves showing diarrhea. Of 23

IFA positive samples, 4 samples (5%) showed cryptosporidial oocysts more than 10(5) **OPG**. Therefore, it is concluded that the calves showing cryptosporidial oocysts more than 10(5) **OPG** in the feces were highly associated with clinical cryptosporidiosis.

L21 ANSWER 10 OF 17 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

95086802 EMBASE Document No.: 1995086802. Toxocara-induced eosinophilic inflammation: Airway function and effect of anti-IL-5. Buijs J.; Egbers M.W.E.C.; Lokhorst W.H.; Savelkoul H.F.J.; Nijkamp F.P.. Laboratory for Parasitology/Mycology, NIPHEP, P.O. Box 1,3720 BA Bilthoven, Netherlands. American Journal of Respiratory and Critical Care Medicine 151/3 I (873-878) 1995.

ISSN: 1073-449X. CODEN: AJCMED. Pub. Country: United States. Language: English. Summary Language: English.

AB The immunoinflammatory response to parasitic nematode infections and allergic diseases have some similarities, the most profound being the increases in eosinophils and serum total IgE concentration. Whether parasitic infections stimulate or inhibit allergic asthma is a matter of debate. We investigated the effect of Toxocara canis (T. canis) infection on airway function in BALB/c mice at various days post-infection. Within 24 h after infection, the trachea responded hyperreactive to carbachol stimulation. Eosinophils, and to a lesser degree lymphocytes, infiltrated the airways causing interstitial and alveolar inflammation (7 d post-infection). Concurrently with cell infiltration, the trachea became hyporesponsive to carbachol whereas the pulmonary resistance was increased and the dynamic compliance decreased. The hyporeactive response could be simulated in vitro by incubating normal tracheae with eosinophil-enriched bronchoalveolar lavage cells obtained from infected mice. The response depended on the number of cells added to the medium, a lower number causing a hyper- and a higher number a hyporeactive response. Anti-interleukin-5 (anti-IL-5) producing hybridoma cells given simultaneously with T. canis infection inhibited eosinophil infiltration in the airways but not that of lymphocytes. Anti-1L- 5 treatment prevented tracheal hyporeactivity but not perivascular and peribronchial edema, increased pulmonary resistance, or decreased dynamic compliance. Treatment with isotype control **antibody** did not affect eosinophil number nor the observed changes in airway functions. It was concluded that T. canis-induced airway inflammation coincided with increased pulmonary resistance, decreased dynamic compliance, and perivascular/peribronchial edema. These phenomena were independent on the presence of eosinophils, whereas tracheal hyporeactivity was clearly associated with airway eosinophilia.

L21 ANSWER 11 OF 17 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

95184483 EMBASE Document No.: 1995184483. Behavioral studies on the putative

.gamma.-type endorphin receptor using different **antibodies**. Van Ree J.M.; Wolterink G.; Igarashi Y.; Vanderschuren L.; Wiegant V.M.; Rust C.J.J.; Bruning H.W.. Department of Pharmacology, Rudolf Magnus Institute Neuroscience, Utrecht University, Universiteitsweg 100,3584 CG Utrecht, Netherlands. European Journal of Pharmacology 279/2-3 (187-196) 1995. ISSN: 0014-2999. CODEN: EJPHAZ. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB To investigate the significance of endogenous, neuroleptic-like .gamma.-type endorphins and their putative receptors, polyclonal and monoclonal **antibodies** against .gamma.-type endorphins, which may bio-inactivate the ligands for the receptors, and monoclonal anti-idiotype **antibodies**, which presumably bind to the receptors, were injected into the nucleus accumbens of the rat brain. The desenkephalin-.gamma.-endorphin-induced antagonism of the hypomotility response elicited by challenge with apomorphine injected into the nucleus accumbens was used as test system. Both the anti-desenkephalin-.gamma.-endorphin **antibodies** and anti-idiotype **antibodies** blocked the action of exogenous desenkephalin-.gamma.-endorphin. Thus, the anti-idiotype **antibodies** may serve as receptor antagonists. Chronic treatment (injection into the nucleus accumbens) with the anti-idiotype **antibodies** induced sustained hypermotility, decreased habituation and impaired passive avoidance behavior. In such treated animals local treatment with apomorphine did not elicit hypomotility. It is suggested that .gamma.-type endorphins influence the setpoint for feedback regulation in dopaminergic neurons equipped with .gamma.-type endorphin receptor systems.

L21 ANSWER 12 OF 17 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.

93189635 EMBASE Document No.: 1993189635. Topography and characteristics of specific binding sites for non-opioid .gamma.-type endorphins in the rat brain as studied by autoradiography with [35S]Met-desenkephalin-.gamma.-endorphin. Ronken E.; Wiegant V.M.; Kaspersen F.M.; Van Nispen J.W.; De Boer T.; Bruning H.W.; Rust C.J.J.; Tonnaer J.A.D.M.. Department of Pharmacology, Medical Faculty, Free University, Van der Boechorststraat 7,1081 BT Amsterdam, Netherlands. Brain Research 615/1 (63-70) 1993. ISSN: 0006-8993. CODEN: BRREAP. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB An in vitro autoradiographic study was performed to characterize specific rat brain binding sites for non-opioid neuroleptic-like .gamma.-type endorphins, using [35S]Met-des-enkephalin-.gamma.-endorphin ([35S]Met-DE.gamma.E; [35]S-.beta.-endorphin(5-17)) with high specific activity as radioligand. The binding sites appeared to be confined to rat forebrain regions, e.g., orbital cortex, frontal cortex, cingulate cortex, piriform cortex, nucleus accumbens, amygdala, mediodorsal nucleus of the thalamus and arcuate and periventricular nuclei of the hypothalamus. These regions are part of the mesocorticolimbic feedback circuit. Densitometric analysis of the autoradiographs revealed that the density of the binding sites was highest in the mediodorsal nucleus of the thalamus and the amygdala. Concentration-dependent displacement of [35S]Met-DE.gamma.E (500 pM) with DE.gamma.E yielded an IC50 of 0.6 nM whereas DE.alpha.E (.beta.-endorphin(6-16)) had an IC50 of 210 nM. Various endorphins, sharing the .gamma.-endorphin C terminus, displaced [35S]Met-DE.gamma.E to the same extent as non-labelled DE.gamma.E (at 10-6M) whereas non-endorphin peptides did not show displacing capacity. Possible relationships of the binding sites with opioid receptors were

investigated. DAMGO (.mu.) and DPDPE (.delta.) displaced [35S]Met-DE.gamma.E to some extent at 10-6M whereas U69,593 (.kappa.) was inactive, suggesting that the binding sites for .gamma.-type endorphins may resemble .mu.- and .delta.-opioid receptors in some aspects. Similarly, relationships with dopamine receptors were investigated. Haloperidol partially displaced [35S]Met-DE.gamma.E whereas sulpiride, SKF38,393 and 3-PPP at 10-6M did not induce significant displacement. Thus, binding sites are distinct from dopamine receptors. Finally, the monoclonal anti-DE.gamma.E anti-idiotypic **antibody** CR14.1 appeared to be a potent competitor of [35S]Met-DE.gamma.E. The results obtained indicate that [35S]Met-DE.gamma.E labels a specific class of binding sites in the brain. These binding sites are selective for .gamma.-type endorphins, and are distinct from opioid and dopamine receptors. In view of their topography and binding characteristics, the binding sites for .gamma.-type endorphins may be of relevance for the neuroleptic-like activity of these peptides.

L21 ANSWER 13 OF 17 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
91217797 EMBASE Document No.: 1991217797. The influence of Org 10172, a low molecular weight heparinoid, on antipyrine metabolism and the effect of enzyme induction on the response to Org 10172. De Boer A.; Stiekema J.C.J.; Danhof M.; Breimer D.D.. Centre for Human Drug Research, P.O. Box 9600,2300 RC Leiden, Netherlands. British Journal of Clinical Pharmacology
32/1 (23-29) 1991.
ISSN: 0306-5251. CODEN: BCPHBM. Pub. Country: United Kingdom. Language: English. Summary Language: English.
AB 1. We have investigated the effect of repeated s.c. Org 10172 (a low molecular weight heparinoid; Lomoparan.RTM.) treatment (1000 anti-Xa units twice daily for 5 days) on antipyrine (500 mg orally) metabolism, and the effect of enzyme induction by pentobarbitone (100 mg for 12 days) on the pharmacokinetics and pharmacodynamics of Org 10172 following an intravenous bolus injection of 3250 anti-Xa units. 2. Org 10172 treatment caused a small increase in the formation rates of all antipyrine metabolites ($P < 0.05$), while the overall kinetics of antipyrine did not change significantly. 3. Oxidative enzyme induction by pentobarbitone, as demonstrated by an increased clearance of antipyrine, was associated with an increase in the area under the anti-thrombin activity vs time curve ($P < 0.05$). No influence was seen on the kinetics of plasma anti-Xa and thrombin generation inhibiting (TGI) activity. 4. The pharmacodynamics of Org 10172, as determined by clotting tests, was not influenced by enzyme induction. 5. The clinical relevance of these observations is likely to be limited.

L21 ANSWER 14 OF 17 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
88197438 EMBASE Document No.: 1988197438. Vasopressin and adrenalectomy-induced sensitivity to morphine. Ratka A.; De Kloet E.R.. Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, 3521 GD Utrecht, Netherlands. European Journal of Pharmacology
153/1 (65-71) 1988.
ISSN: 0014-2999. CODEN: EJPHAZ. Pub. Country: Netherlands. Language: English. Summary Language: English.
AB Arginine vasopressin, vasopressin antiserum and a specific vasopressin pressor antagonist were injected intracerebroventricularly into adrenalectomized rats before morphine-induced antinociception was tested. In these experiments we have exploited previous findings which showed that the antinociceptive effect of opioids was potentiated after adrenalectomy;

rats that were adrenalectomized in the morning under basal resting conditions of the pituitary-adrenal system displayed significantly higher response latencies after morphine administration than rats adrenalectomized in the evening. These effects were measured 7 days after adrenalectomy. The same conditions were used in this study. Both, the vasopressin antiserum and the vasopressin antagonist abolished the morning adrenalectomy-induced hypersensitivity to centrally injected morphine and were not effective when administered to rats that had been adrenalectomized in the evening. The reverse was observed after intraventricular administration of vasopressin. The peptide significantly raised the sensitivity to morphine-induced antinociception of rats that had been adrenalectomized in the evening whereas it did not affect antinociception in animals that had been adrenalectomized in the morning. Vasopressin levels determined by radioimmunoassay in the cerebrospinal fluid were significantly higher in adrenalectomized animals. We propose that vasopressin is a critical neuropeptide factor involved in the adrenalectomy-induced hypersensitivity to morphine antinociception.

L21 ANSWER 15 OF 17 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
87037272 EMBASE Document No.: 1987037272. Anterior hypothalamic lesions decrease anaphylactic contractions in guinea pig trachea in vitro by reducing histamine and LTC4 reactivity. Van Oosterhout A.J.M.; Nijkamp F.P.. Institute of Veterinary Pharmacology, Pharmacy and Toxicology, University of Utrecht, 3508 TD Utrecht, Netherlands. International Journal of Immunopharmacology 8/8 (975-983) 1986.
CODEN: IJIMDS. Pub. Country: United Kingdom. Language: English.
AB Discrete lesions in the anterior hypothalamus (AHA) of the guinea pig brain reduce the anaphylactic contraction of the trachea in vitro after active in vivo sensitization by 40%. This difference in anaphylactic contraction does not correlate with a difference in homocytotropic **antibodies** but coincides with a decreased smooth muscle response to the anaphylactic mediators histamine and leukotriene C4. No difference in the .beta.-adrenoceptor function of the tracheal preparations can be found. The results suggest that AHA lesions afford protection against anaphylaxis in actively sensitized guinea pigs at least in part through a reduced smooth muscle response to anaphylactic mediators.

L21 ANSWER 16 OF 17 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
79025521 EMBASE Document No.: 1979025521. Adrenocorticotrophic hormone fragments mimic the effect of morphine in vitro. Plomp G.J.J.; Van Ree J.M.. Cent. Hum. Toxicol., Univ. Utrecht, Netherlands. British Journal of Pharmacology 64/2 (223-227) 1978.
CODEN: BJPCBM. Pub. Country: United Kingdom. Language: English.
AB Fragments of the N terminal part of adrenocorticotrophic hormone (ACTH) inhibited the electrically evoked contractions of the mouse vas deferens. This inhibition could be antagonized by naloxone. The same fragments displaced radiolabelled morphine antiserum. Structure activity relationship studies showed that in both assay systems the active core is located within the sequence ACTH 7-10. It is postulated that the Trp9 residue and the peptide bond between Trp9 and Gly10 are particularly important for interaction of ACTH fragments with morphine receptors.

L21 ANSWER 17 OF 17 MEDLINE DUPLICATE 4
78129063 Document Number: 78129063. [Comparative study on the immunogenicity of plasma substitutes with gelatin base in animal experiments]. Vergleichende Studie uber die Immunogenitat von Plasmaersatzlosungen auf Gelatinebasis im Tierexperiment. Sonneborn H H; De Weck A L; Toffler O. INFUSIONSTHERAPIE UND KLINISCHE ERNAHRUNG, (1978 Feb) 5 (1) 41-9. Journal

code: GOI. ISSN: 0378-0791. Pub. country: Switzerland. Language: German.

AB The immune response of rabbits and guinea pigs to gelatin and to three commercial plasma substitutes based on gelatin (oxypolygelatin [**OPG**], di-isocyanate cross-linked gelatin [DCG], modified fluid gelatin [MFG]) has been reevaluated. Passive hemagglutination, passive cutaneous anaphylaxis and active anaphylaxis have been used to detect the response of animals immunized in complete Fruend's adjuvant or in aluminium hydroxide. Marked immune responses were observed with DCG and MFG which were to a large extent specific for the immunizing antigen (i.e. the corresponding chemically modified gelatin). Gelatin and **OPG** induced weak responses. In contrast to DCG and MFG in systemic anaphylactic shock experiments no anaphylactic shock could be elicited with gelatin and **OPG**. A limited series of immunizations in guinea pigs of various strains demonstrated that responses to weak immunogens, such as modified gelatins, are markedly influenced by genetic factors.

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